MOLECULAR AND CELLULAR BIOLOGY, June 2005, p. 4377–4387 Vol. 25, No. 11
0270-7306/05/$08.00+0 doi:10.1128/MCB.25.11.4377–4387.2005

Fission Yeast Rad51 and Dmc1, Two Efficient DNA Recombinases Forming Helical Nucleoprotein Filaments

Synthia Sauvageau,[1] Alicja Z. Stasiak,[2] Isabelle Banville,[1] Mickaël Ploquin,[1] Andrzej Stasiak,[2] and Jean-Yves Masson[1]*

Genome Stability Laboratory, Laval University Cancer Research Center, Hôtel-Dieu de Québec, 9 McMahon, Québec City, Quebec G1R 2J6, Canada,[1] and Laboratoire d'Analyse Structurale, Université de Lausanne, 1015 Lausanne, Switzerland[2]

Received 28 January 2005/Returned for modification 2 March 2005/Accepted 8 March 2005

Homologous recombination is important for the repair of double-strand breaks during meiosis. Eukaryotic cells require two homologs of *Escherichia coli* RecA protein, Rad51 and Dmc1, for meiotic recombination. To date, it is not clear, at the biochemical level, why two homologs of RecA are necessary during meiosis. To gain insight into this, we purified *Schizosaccharomyces pombe* Rad51 and Dmc1 to homogeneity. Purified Rad51 and Dmc1 form homo-oligomers, bind single-stranded DNA preferentially, and exhibit DNA-stimulated ATPase activity. Both Rad51 and Dmc1 promote the renaturation of complementary single-stranded DNA. Importantly, Rad51 and Dmc1 proteins catalyze ATP-dependent strand exchange reactions with homologous duplex DNA. Electron microscopy reveals that both *S. pombe* Rad51 and Dmc1 form nucleoprotein filaments. Rad51 formed helical nucleoprotein filaments on single-stranded DNA, whereas Dmc1 was found in two forms, as helical filaments and also as stacked rings. These results demonstrate that Rad51 and Dmc1 are both efficient recombinases in lower eukaryotes and reveal closer functional and structural similarities between the meiotic recombinase Dmc1 and Rad51. The DNA strand exchange activity of both Rad51 and Dmc1 is most likely critical for proper meiotic DNA double-strand break repair in lower eukaryotes.

Meiosis generates haploid gametes or spores in yeast through a cell division process that consists of one round of DNA replication followed by two cell divisions. These divisions are preceded by a unique meiotic prophase during which two prominent events occur. Homologous chromosomes synapse in a tripartite proteinaceous structure called the synaptonemal complex and undergo genetic recombination at frequencies that are 100- to 1,000-fold higher than in vegetative cells. Reciprocal recombination events, named crossovers, establish chiasmata, which are physical connections between homologues that ensure proper chromosome segregation during meiosis I. Meiosis II involves the segregation of sister chromatids and is therefore analogous to a mitotic division. The consequences and benefits of meiotic interhomolog recombination are correct chromosome segregation, gene rearrangements, and genetic diversity (35).

Current knowledge indicates that the mechanism of meiotic recombination is analogous to the process by which ionizing radiation induced double-strand breaks (DSBs) are repaired. Meiotic DNA breakage in *Schizosaccharomyces pombe* requires multiple gene products. In *S. pombe*, the Rec6, Rec7, Rec12, Rec14, and Rec15 proteins are essential for the creation of DNA DSBs and meiotic recombination (12, 16). A key component for DSB formation in all known organisms is Spo11 (Rec12 in *S. pombe*), a type II topoisomerase (30). Spo11 knockout mice display chromosome synapsis defects, suggesting that the initiation of recombination precedes and is required for normal synapsis of chromosomes during meiosis I (2, 46). Resection of the DSB involves the MRE11, RAD50, and XRS2 gene products, giving rise to recombinogenic 3′ single-stranded tailed DNA (50). The resected DSBs are then used to invade homologous duplex DNA leading to the exchange of genetic information, a process that requires the RAD51 and DMC1 genes.

Rad51 and meiosis-specific protein Dmc1 are homologs of bacterial recombinase RecA (8, 9, 23). The DMC1 gene was originally identified in *Saccharomyces cerevisiae* by using a screen for meiosis-specific prophase-induced genes that cause a meiotic defect when disrupted (9). Mutations in *S. cerevisiae* DMC1 lead to the accumulation of resected DSBs and prevention to complete meiosis in some strains (9, 45). Dmc1 mutants also show strong defects in strand invasion, as indicated by the formation of single-strand invasions and double Holliday junctions (28, 47). In *S. pombe*, transcription of the *dmc1+* gene is induced during meiosis (19). Partial or complete deletion of Dmc1 does not affect vegetative growth and leads to a three- to fivefold reduction in recombination frequencies (19, 20). Analysis of homozygous female or male mouse DMC1$^{-/-}$ knockouts revealed that both males and females are sterile. In Dmc1-deficient spermatocyte nuclei, chromosomes show extensive asynapsis despite axial element formation (43, 64). To date, the genetic influence of Rad51 in mouse meiosis cannot be determined due to embryonic lethality. However, it is clear that disruption of the RAD51 gene in vertebrate mitotic cells leads to chromosomal aberrations in the absence of DNA damage (33, 58). In addition, for a key function in homologous recombination, a role for Rad51 in tumor suppression is proposed by the interaction with the breast and ovarian tumor suppressor BRCA2 (15).

* Corresponding author. Mailing address: Genome Stability Laboratory, Laval University Cancer Research Center, Hôtel-Dieu de Québec, 9 McMahon, Quebec City, Quebec G1R 2J6, Canada. Phone: 418-525-4444, ext. 15154. Fax: 418-691-5439. E-mail: Jean-Yves.Masson@crhdq.ulaval.ca.

Although considerable interest has been devoted to biochemical studies of Rad51 proteins, only little is known of its homolog Dmc1. The DNA-binding domain of tumor suppressor protein p53 has been found to associate with the C-terminal domain of mouse Dmc1. The functional consequence of this interaction is not clear, but one possibility is that the association between p53 and Dmc1 might help to control homologous recombination in meiotic germ cells (24). Physical interactions have been observed between human Rad51 and Dmc1 with the MSH4 protein, a protein required for synapsis and the wild-type level of meiotic crossovers (39). In *Arabidopsis*, Brca2 interacts with Dmc1 and Rad51, suggesting an important role for Brca2 in the control of homologous recombination at meiosis (49). Dmc1 proteins possess features reminiscent of RecA/Rad51, since they contain Walker A and B boxes for ATP hydrolysis (42). In RecA, the disordered loops L1 and L2 are proposed to be involved in binding of single- and double-stranded DNA (54). Loop 1 is well conserved in Dmc1 proteins, although the sequence conservation of Loop 2 is less pronounced (42). *S. cerevisiae* Dmc1 and human Dmc1 proteins have been purified to homogeneity (26, 32, 34). Human Dmc1 has been shown to form octameric rings that can bind DNA as a stack (34, 41). The octameric ring structure of the human Dmc1 ring has been solved at a 3.2-Å resolution (31). Recent studies show that human Dmc1 also forms nucleoprotein filaments (48).

Since its discovery in 1992, budding yeast Dmc1 has been functionally related to Rad51. However, in contrast to Rad51 and despite the high degree of similarity at the amino acid level, *S. cerevisiae* Dmc1 was poorly efficient in promoting homologous recombination in vitro since the maximum level reached in D-loop formation was 1% (26). To date, helical filaments of *S. cerevisiae* Dmc1 have not been detected which contrast with genetic studies, suggesting an important role for Dmc1 in homologous recombination during meiosis. In order to investigate whether this discrepancy between genetics and biochemistry was conserved during evolution, we purified *S. pombe* Rad51 and Dmc1. We demonstrate here that fission yeast Dmc1, like Rad51, can form helical nucleoprotein filaments and promote strand exchange with homologous DNA, revealing a closer structural and functional relationship between these proteins.

MATERIALS AND METHODS

Plasmids and DNA. Single-stranded ϕX174 DNA was purchased from New England Biolabs. pPB4.3 (6) and pJYMAT4.2 Single-stranded (ssDNA) (34) were prepared by standard protocols. Linear duplex DNA with 1.2-kb 3′ single-stranded tails was produced by annealing EcoRI- and BamHI-linearized pDEA-7Z to pJYMAT4.2 ssDNA as described previously (34). Linear duplex DNA with 3′ single-stranded tails at both ends were prepared as described previously (37). All DNA concentrations are expressed in moles of nucleotides.

The *spdmc1*+ gene was PCR amplified from *S. pombe* genomic DNA (kindly provided by H. Yamano, CRUK) with primers bearing NdeI and BamHI sites. The PCR product was subjected to partial digestion with NdeI (because *spdmc1*+ bears an internal NdeI site) and cloned into pET16b (Novagen) to generate pSpDmc1-16b in which the Dmc1 protein sequence was linked to a decahistidine tag. An intronless derivative of SpDmc1 was generated by removing a 74-bp intron by using the primers JYM74 and JYM75 essentially as described by Wang and Malcolm (60). The final nucleotide sequence was identical to gene accession number AB008545. The *sprad51*+ gene was amplified by PCR from a mitotic *S. pombe* cDNA library (kindly provided by H. Yamano, CRUK) with primers bearing NdeI and BamHI sites. The PCR product was subjected to partial digestion with NdeI (because *sprad51*+ bears an internal NdeI site) and cloned into pET16b (Novagen) to generate pSpRad51-16b. The sequence of S. pombe *rad51*+ was identical to accession number D13805.

Purification of *S. pombe* Dmc1 and Rad51 proteins. Recombinant SpDmc1 protein was purified from 8 liters of *E. coli* FB850 *recA* mutant pLysS (7) carrying plasmid pSpDmc1-16b grown at 37°C in tryptone phosphate medium supplemented with 100 μg of ampicillin/ml and 25 μg of chloramphenicol/ml. At an optical density at 600 nm (OD_{600}) of 0.5, SpDmc1 synthesis was induced by the addition of 0.05 mM IPTG (isopropyl-β-D-thiogalactopyranoside) and incubated at 30°C; after 4 h, the cells were harvested by centrifugation, frozen in dry ice, and stored at −80°C. The cell paste was resuspended in 180 ml of lysis buffer (0.1 M Tris-Cl [pH 8.0], 2 mM EDTA, 5% glycerol, 0.5 mM dithiothreitol [DTT], 0.1% Triton X-100) containing the protease inhibitors phenylmethylsulfonyl fluoride (1 mM), aprotinin (0.019 trypsin inhibitor unit [TIU]/ml), leupeptin (1 μg/ml), and pepstatin A (1 μg/ml). The suspension was divided into 40-ml aliquots, which were sonicated three times for 30 s. Insoluble material was removed by centrifugation at 40,000 rpm for 1 h in a Sorvall Ultra Pro 80 T647.5 rotor. The supernatant was dialyzed three times against 4.5 liters of spermidine buffer (20 mM Tris-acetate [pH 7.5], 7 mM spermidine-NaOH [pH 7.5], 0.1 mM DTT) for 8 h at 4°C. The precipitate containing Dmc1 was recovered by centrifugation, and the pellet was resuspended in 120 ml of T5 buffer (20 mM Tris-Cl [pH 8.0], 0.5 M NaCl, 5% glycerol, 5 mM imidazole, 0.02% Triton X-100) containing the protease inhibitors aprotinin (0.019 TIU/ml) and leupeptin (1 μg/ml). Insoluble materials was removed by centrifugation (20,000 rpm for 20 min at 4°C in a Sorvall Ultra Pro 80 T647.5 rotor).

The supernatant was loaded on a 20-ml Talon column (BD Biosciences). The column was washed successively with 200 and 160 ml of T buffer containing 30 and 40 mM imidazole, respectively, before Dmc1 was eluted with a 160-ml linear gradient of 0.04 to 0.8 M imidazole in T buffer. Fractions of Dmc1, which eluted as a broad peak, were identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), pooled, and dialyzed against R buffer (50 mM Tris-HCl [pH 8.0], 10% glycerol, 0.5 mM DTT) containing 150 mM KCl (R150) and loaded onto a 20-ml Heparin Fastflow column (Pharmacia). The column was washed with 200 ml of R150, and Dmc1 was eluted with a 160-ml linear gradient of 0.15 to 0.7 M KCl in R buffer. Dmc1, found in the flowthrough, was loaded directly onto a 1-ml MonoQ column (HR 5/5) by using a Pharmacia FPLC system. The column was washed with 20 ml of R150 before the SpDmc1 was eluted by using a linear gradient of 10 ml of 0.15 to 1.0 M KCl in R buffer. Dmc1 eluted in a sharp peak at 0.5 M KCl. The protein was dialyzed against 1 liter of S buffer (20 mM Tris-acetate [pH 7.5], 0.25 M sodium chloride, 10% glycerol, 0.5 mM DTT) and stored in aliquots at −80°C. The concentration of Dmc1 was determined by SDS-PAGE using purified RecA as a standard. The concentration was also verified by Bradford assay.

S. pombe Rad51 was purified essentially as described for Dmc1 with the following modifications. Recombinant SpRad51 protein was purified from 8 liters of *Escherichia coli* FB850 *recA* pLysS (7) carrying plasmid pSpRad51-16b grown at 37°C in Luria broth supplemented with 100 μg of ampicillin/ml and 25 μg of chloramphenicol/ml. At an OD_{600} of 0.5, SpRad51 synthesis was induced by the addition of 0.1 mM IPTG, followed by incubation at 37°C for 4 h. During the Talon heparin and MonoQ chromatography, the buffers contained 90 mM Tris-Cl instead of 20 mM.

Purification of human Dmc1 and *E. coli* SSB. *Homo sapiens* Dmc1 was purified as described previously (34). Purified *E. coli* single-strand binding protein (SSB) was purchased from USB.

Gel filtration. The molecular mass of purified Rad51 (20 μg) and Dmc1 protein (30 μg) was determined by comparison with gel filtration standards (200 μg; bovine thyroglobulin [670 kDa], bovine gamma globulin [158 kDa], chicken ovalbumin [44 kDa], horse myoglobin [17 kDa], and vitamin B_{12} [1.35 kDa]). Proteins were analyzed on an FPLC Explorer 10 system fitted with a 24-ml Superdex 200 PC 3.2/30 column (Pharmacia) equilibrated in R150 buffer. Fractions were collected and analyzed by SDS-PAGE, followed by Western blotting with a monoclonal anti-histidine antibody diluted at 1/5,000 (BD Biosciences).

Yeast two-hybrid analysis. The full-length *rad51*+ and *dmc1*+ genes were cloned into pGBK-T7 and pGAD-T7 (BD Biosciences) to produce fusions to the Gal4 DNA-binding and activation domains. Human Dmc1 was cloned in the pGAD-C3 vector as described previously (29, 34). Plasmids $pSPDMC1_{1-111}$, $pSPDMC1_{112-218}$, and $pSPDMC1_{219-333}$ were constructed by cloning the appropriate PCR products in pGBK-T7. All fusions were confirmed by sequencing. AH109 and Y187 strains were transformed with the indicated plasmids. Colony growth on medium lacking tryptophan and leucine was observed with all constructions tested here. Interactions between partners were assayed by growth on synthetic medium lacking tryptophan, leucine, adenine, and histidine supplemented with 2.5 mM 3-aminotriazole. Transformation, colony lift assays, and

β-galactosidase (β-Gal) assays were carried out according to the Matchmaker kit manual (BD Biosciences).

DNA-binding assay. Reactions (10 μl) contained 50 nM DNA in binding buffer [20 mM triethanolamine-HCl (pH 7.5), 2 mM ATP, 2.5 mM $Mg(CH_3COO)_2$, 1 mM DTT, and 100 μg of bovine serum albumin (BSA)/ml]. After 5 min at 37°C, the indicated amount of Dmc1 was added (2 μl) and the incubation was continued for a further 5 min. Complexes were fixed by the addition of 0.2% glutaraldehyde, followed by a 15-min incubation at 37°C. Protein-DNA complexes were analyzed by 6% PAGE with Tris-borate-EDTA (TBE) buffer. The gels were dried on DE81 filter paper, followed by autoradiography. DNA substrates were prepared by annealing a ^{32}P-labeled oligonucleotide (100 nucleotides in length) with appropriate complementary sequences. The 100-mer double-stranded, 5′-tailed and 3′-tailed DNA (containing both 50 nucleotides of ssDNA) were purified by 10% PAGE. The sequence of the 100-mer is 5′-GGGCGAATTGGGCCCGACGTCGCATGCTCCTCTAGACTCGAGG AATTCGGTACCCCGGGTTCGAAATCGATAAGCTTACAGTCTCCATTT AAAGGACAAG-3′. DNA concentrations are expressed as moles of DNA molecules.

ATPase assays. Reactions (50 μl) contained excess pPB4.3 ssDNA or form I double-stranded DNA (dsDNA; 150 μM) and 1.5 μM Rad51 or Dmc1 in 50 mM triethanolamine-acetate (pH 7.5), 1 mM $Mg(CH_3COO)_2$, 1 mM DTT, and 100 μg of BSA/ml supplemented with 50 nCi of [γ-^{32}P]ATP (3,000 Ci/mmol; Perkin-Elmer Life Sciences). Aliquots (5 μl) were removed at the indicated times and stopped by the addition of EDTA, and the percentage ATP hydrolyzed was determined by thin-layer chromatography, followed by quantification by using a Storm 860 PhosphorImager (Molecular Dynamics).

Single-strand annealing reactions. Reactions (10 μl) contained denatured 5′-end-labeled pPB4.3 400-bp NdeI-HindIII (450 nM) with Rad51 (400 nM) or Dmc1 (400 nM) in HEPES buffer [20 mM HEPES (pH 7.5), 5 mM $Mg(CH_3COO)_2$ (or the indicated concentration), 2 mM ATP, 1 mM DTT, 100 mM NaCl, and 100 μg of BSA/ml]. Incubation was at 20°C for 5 min or the indicated time. The reaction products were deproteinized by the addition of a one-tenth volume of stop buffer (10% SDS and 10 mg of proteinase K/ml), followed by a 15-min incubation at 20°C. Labeled DNA products were analyzed by electrophoresis through a 4% 1× TBE-PAGE gel run at 150 V for 2 h 15 min, dried onto DE81 filter paper, and visualized by autoradiography.

Strand exchange reactions. Reactions (10 μl) contained purified single-stranded pPB4.3 DNA (15 μM) with the indicated concentrations of Rad51 in standard buffer [50 mM triethanolamine-HCl (pH 7.5), 1 mM $Mg(CH_3COO)_2$, 2 mM ATP, 1 mM DTT, 100 mM NaCl, 100 μg of BSA/ml]. Dmc1 reactions were performed in (35 mM Tris-HCl [pH 7.5], 2 mM $MgCl_2$, 1 mM DTT, 8 mM creatine phosphate, 5 U of phosphocreatine/ml) with or without 1 μM SSB. After 5 min at 37°C, ^{32}P-end-labeled pPB4.3 DNA (a 400-bp fragment) was added, and incubation was continued for 90 min. Reaction products were deproteinized by the addition of one-fifth volume of stop buffer (0.1 M Tris-HCl [pH 7.5], 0.1 M $MgCl_2$, 3% SDS, 5 μg of ethidium bromide/ml, and 10 mg of proteinase K/ml), followed by 45 min incubation at 37°C. Labeled DNA products were analyzed by electrophoresis through 0.8% TAE agarose gels containing 1 μg of ethidium bromide/ml, run at 4.3 V/cm, dried onto DE81 filter paper, and visualized by autoradiography.

Electron microscopy. Rad51 and Dmc1 reactions (10 μl) contained φX174 single-stranded DNA, pPB4.3 tailed DNA, and JYMAT4.2 tailed DNA in 20 mM triethanolamine-HCl (pH 7.5), 1 mM DTT, 2 mM ATP, 2.5 mM $Mg(CH_3COO)_2$. Dmc1 reactions were performed also in 35 mM Tris-HCl (pH 7.5), 2 mM $MgCl_2$, 1 mM DTT, and 2 mM ATP. After 5 min at 37°C, the indicated amounts of protein was added, and incubation was continued for a further 10 min. When fixation was required, protein-DNA complexes were fixed by the addition of glutaraldehyde to 0.2%, followed by 15 min of incubation at 37°C. Samples were diluted and washed in 5 mM $Mg(CH_3COO)_2$ prior to uranyl acetate staining (51). Complexes were visualized at a magnification of ×20,500 by using a Philips CM100 electron microscope.

RESULTS

Expression and purification of *S. pombe* Rad51 and Dmc1. The *rad51*+ and *dmc1*+ genes were PCR amplified from a *S. pombe* mitotic cDNA library and genomic DNA library, respectively. The sequence of *S. pombe rad51*+ was identical to accession number D13805. The Dmc1 PCR product was recovered, and the genomic intron was removed by using a modified QuikChange site-directed mutagenesis protocol (60). The corresponding cDNA was identical to the published sequence of Fukushima et al. (19). *rad51*+ and *dmc1*+ were cloned into pET16b (Novagen) to place a 10-histidine tag at the N termini of the proteins. Recombinant Rad51 and Dmc1 proteins were overexpressed and purified from an *E. coli* strain carrying a deletion of the *recA* gene to avoid possible contamination with RecA. After induction by IPTG, Rad51 was precipitated selectively by spermidine, and the solubilized protein was then subjected to Talon affinity chromatography. Rad51 was essentially pure after this step, but minor contaminants were removed by heparin and MonoQ chromatography. Dmc1 was purified with the same chromatographic steps. The final elutions of Rad51 and Dmc1 from the MonoQ column are shown in Fig. 1A and B, respectively. The proteins were free of endo- and exonuclease activities (data not shown).

Gel filtration and self-interactions by *S. pombe* Rad51 and Dmc1. The native molecular masses of Rad51 and Dmc1 were determined by gel filtration through Superdex 200. Both Rad51 and Dmc1 eluted with a broad profile, suggesting that these proteins could form complexes containing a variable number of molecules. However, the bulk of Rad51 and Dmc1 eluted in fractions 18 to 20 (Fig. 1D) or in fractions 21 and 22 (Fig. 1C), respectively. The Dmc1 protein peak corresponded to a native molecular mass of ca. 310 kDa. Since the predicted molecular mass of his-tagged Dmc1 is 38,944 Da, this is indicative of an octameric form. Similarly, the molecular mass of Rad51 was found to be octameric based on the deduced molecular mass of 330 kDa (His-tagged Rad51 has a molecular mass of 42,285 Da). Consistent with this result, human Rad51 (3, 63) and Dmc1 (34, 41) have been shown to form octameric rings. Monomeric Rad51 or Dmc1 were not detected (Fig. 1D, lane 37, and Fig. 1C, lane 30).

Using two-hybrid analysis, the homotypic interactions of Rad51 and Dmc1 were characterized further. Coexpression of full-length Rad51 fused to the Gal4 DNA binding and activation domains in yeast leads to growth on omission media lacking uracil, tryptophan, adenine, and histidine (Fig. 2A). The self-interaction domain of Rad51 is located in the N-terminal part of the protein since the last 115 amino acids of the protein were not required for Rad51-Rad51 interaction. No interaction was observed when Rad51 was divided in three parts (amino acids 1 to 125, 126 to 250, and 251 to 366, respectively) (data not shown). Interestingly, *S. pombe* Rad51 was found to interact with human Rad51, suggesting that critical amino acid residues for self-interaction are conserved between the proteins (Fig. 2A). Coexpression of full-length Dmc1 leads to growth on omission medium and strong β-Gal activity (Fig. 2B and D). Truncations of Dmc1 (amino acids 1 to 111, 112 to 218, and 219 to 333, respectively) revealed that the N terminus of the protein is required for self-interaction (Fig. 2C and D). Further deletions of the protein revealed that the first 55 amino acids are required for self-interaction. Interestingly, full-length *S. pombe* Dmc1 or proteins carrying the first 111 amino acids did not interact with human Dmc1. Consistent with previous studies (11), *S. pombe* Rad51 and Dmc1 do not interact by two-hybrid studies (data not shown).

DNA binding by *S. pombe* Rad51 and Dmc1. In the presence of ATP, RecA binds ssDNA preferentially to dsDNA because of a kinetic barrier to association with dsDNA (44). This difference in affinity is critical for strand exchange reactions since

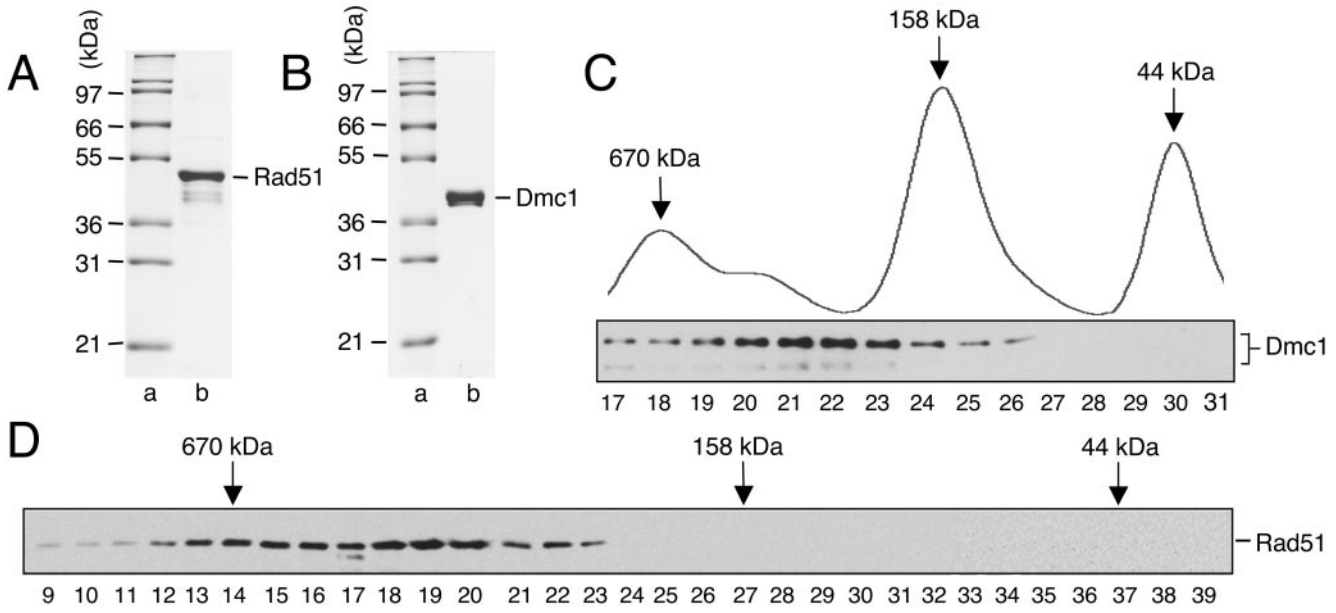

FIG. 1. Purification of fission yeast Rad51 and Dmc1 proteins. (A) SDS-PAGE of purified Rad51. Lane a, molecular weight markers; lane b, purified Rad51 (5 μg). (B) SDS-PAGE of purified Dmc1. Lane a, molecular weight markers; lane b, purified Dmc1 (5 μg). Proteins were visualized with Coomassie blue. (C and D) Multimeric nature of Dmc1 (C) and Rad51 (D). The native molecular mass of purified Dmc1 or Rad51 was determined by gel filtration through Superdex 200. The top part shows size standards; the bottom part shows Western blotting of the indicated proteins in different fractions after elution.

pairing occurs between ssDNA and a naked duplex DNA. Electrophoretic mobility shift assays were used to investigate the ssDNA and dsDNA binding properties of Rad51 and Dmc1. Rad51 exhibited a similar binding pattern to RecA as Rad51 bound preferentially to ssDNA (100 nucleotides in length) over dsDNA (Fig. 3A, compare lanes b to e and g to j). Moreover, 3′-tailed DNA was bound more efficiently than 5′-tailed DNA by Rad51 (lanes l to o and q to t). When purified

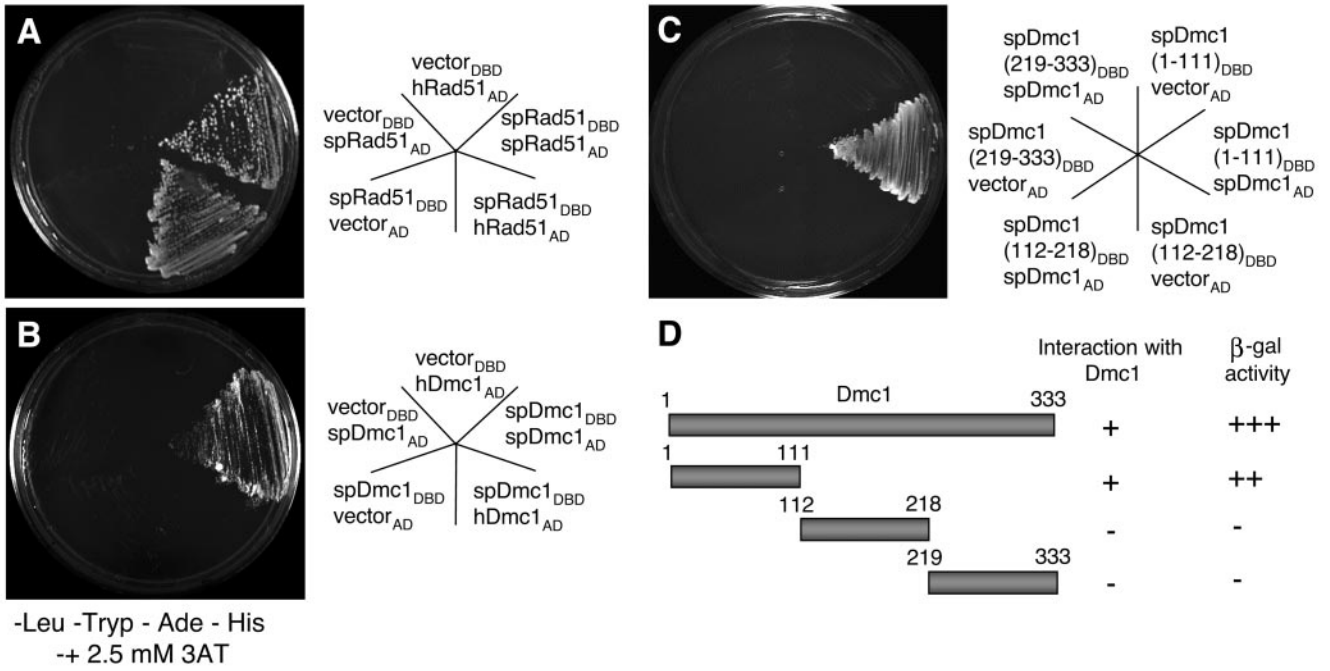

FIG. 2. Two-hybrid analysis of *S. pombe* Rad51 and Dmc1. (A) Fission yeast Rad51 interacts with itself and with human Rad51. (B) Self-association of Dmc1. (C) Mapping of Dmc1 self-interacting domain. AH109 strain was cotransformed with the indicated plasmids, and positive interactions were monitored by growth on medium lacking tryptophan, leucine, adenine, and histidine supplemented with 2.5 mM aminotriazole. The annotations DBD and AD design fusions to the Gal4 DNA-binding domain and Gal4 activation domain, respectively, are indicated. (D) Diagram of the Dmc1 constructs transformed in yeast strain Y187. Positive interactions were measured by β-Gal activity. +++, Highest activity level; −, absence of coloration.

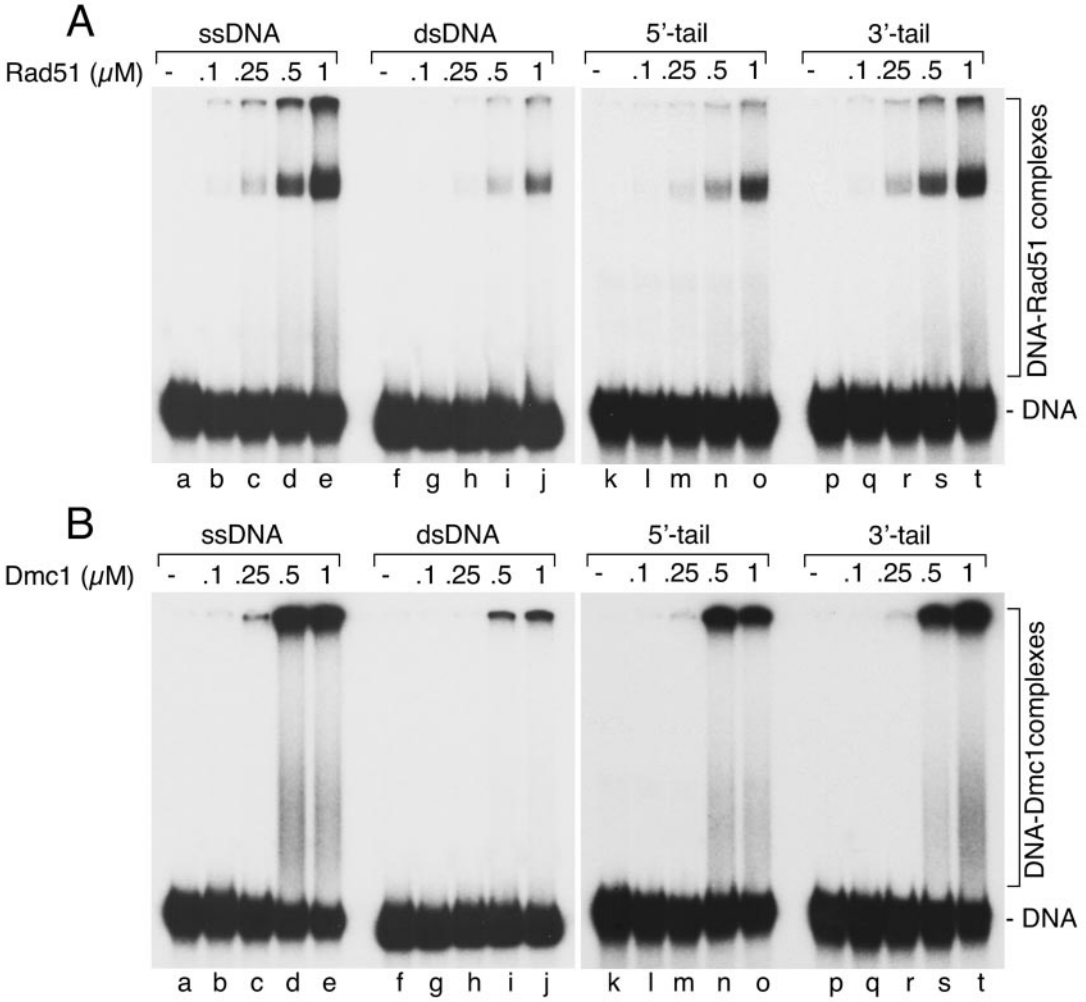

FIG. 3. DNA-binding activity of *S. pombe* Rad51 and Dmc1. DNA-binding reactions contained ssDNA (lanes a to e), dsDNA (lanes f to j), 5′-tailed duplex DNA (lanes k to o), and 3′-tailed duplex DNA (lanes p to t) and the indicated concentrations of Rad51 (A) or Dmc1 (B). Protein-DNA complexes were analyzed by 6% PAGE in TBE buffer.

Dmc1 was incubated with ssDNA, we observed the formation of protein-DNA networks that tended to smear up the gel (Fig. 3B, lanes d and e). This result is characteristic of the formation of networks containing a variable number of protein and DNA molecules. Dmc1 bound preferentially to ssDNA rather than duplex DNA (compare lanes b to e and g to j). Indeed, quantification of the DNA-protein complexes formed with each substrate at 500 nM Dmc1 indicated that only 6% of the input duplex DNA was stably bound compared to 70% for the ssDNA. Dmc1 bound preferentially to 3′-tailed DNA substrates over 5′-tailed DNA substrate (lanes l to o and q to t).

The binding of ssDNA by Rad51 and Dmc1 showed a requirement for Mg^{2+}. Binding to ssDNA was observed both in the absence of ATP or ATP analogs, suggesting that DNA binding can occur without ATP or ATP hydrolysis. The formation of protein-DNA complexes was supported in a wide range of $Mg(CH_3COO)_2$ concentrations (from 1 to 25 mM, with an optimal concentration at 2.5 mM) and was eliminated by inclusion of sodium chloride at concentrations in excess of 200 mM (data not shown).

DNA-stimulated ATPase activity by *S. pombe* Rad51 and Dmc1. Since fission yeast Rad51 and Dmc1 possess Walker A and B amino acid motifs, we sought to determine whether the protein could hydrolyze ATP. Members of the RecA family can hydrolyze ATP in a DNA-stimulated manner. Fission yeast Rad51 and Dmc1 were no exceptions, and the ATP turnover rates on ssDNA were calculated to be 0.7 and 0.6 min^{-1}, respectively. This is in good agreement with the k_{cat} values reported for human Rad51 (0.16 min^{-1}) (4), *S. cerevisiae* Dmc1 (0.7 min^{-1}) (26), and human Dmc1 (1.5 and 0.7 min^{-1}) (32) (22). Rad51 and Dmc1 also hydrolyzed ATP in the presence of dsDNA and to a lesser extent in the absence of DNA (Fig. 4A and B).

Strand annealing and strand exchange catalyzed by Rad51 and Dmc1. Having established conditions under which Rad51 and Dmc1 interact with DNA, we next investigated their ability to promote single-strand annealing. Long ssDNA was used instead of oligonucleotides, which can be prone to artifacts. Interestingly, Rad51 and Dmc1 proteins promote annealing of complementary ssDNAs of 400 nucleotides. Single-strand an-

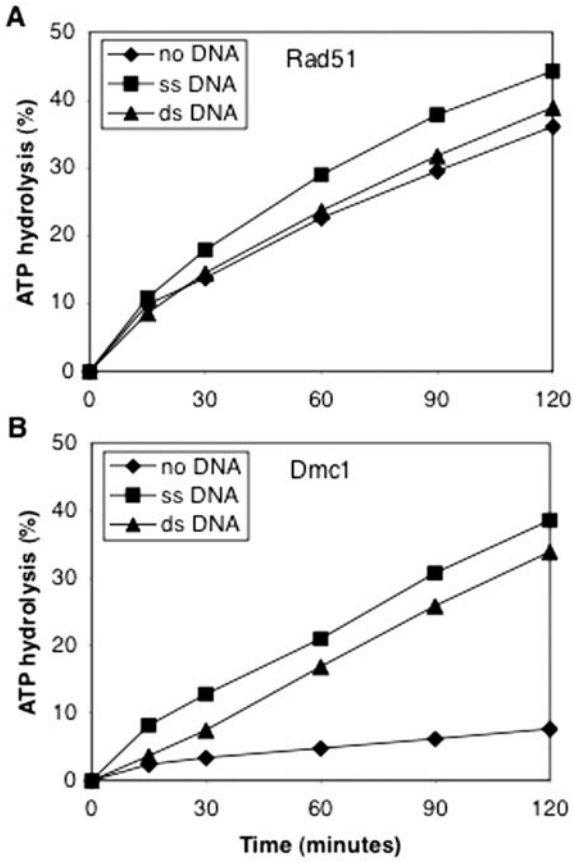

FIG. 4. ATPase activities of *S. pombe* Rad51 and Dmc1. ATP hydrolysis of Rad51 (A) and Dmc1 (B) over time was analyzed in the presence of ssDNA (squares); circular dsDNA (triangles), or the absence of DNA (diamonds), as indicated.

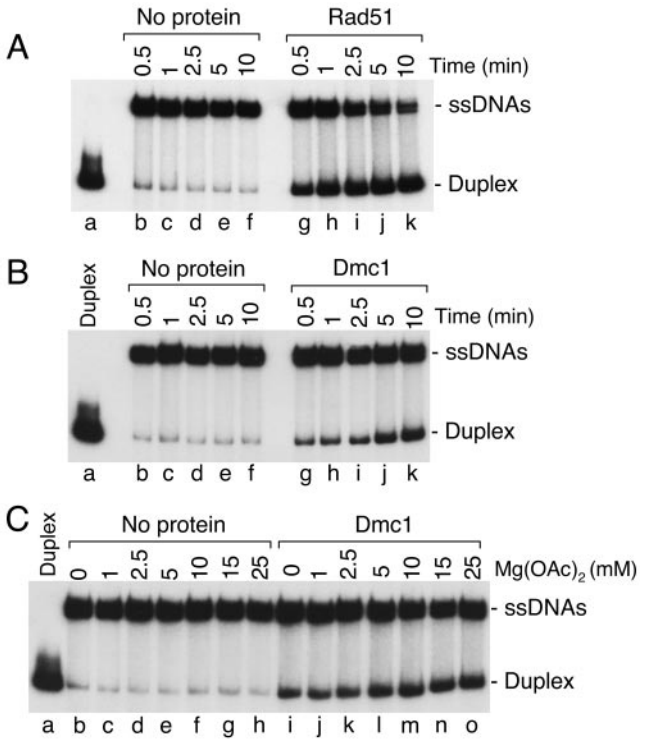

FIG. 5. Single-strand annealing properties of *S. pombe* Rad51 and Dmc1. (A) Time course of Rad51-dependent single-strand annealing. Lane a, purified 400-bp duplex DNA. Reactions contained no protein (lanes b to f) or Rad51 (lanes g to k), and the reaction was stopped at the indicated time. (B) Dmc1 promotes single-strand annealing. Lane a, duplex DNA. Reactions contained no protein (lanes b to f) or Dmc1 (lanes g to k), and the reaction was stopped at the indicated time. (C) Effect of magnesium on Dmc1-dependent single-strand annealing. Lane a, duplex DNA. Reactions contained no protein (lanes b to h) or purified Dmc1 (lanes i to o) and the indicated magnesium acetate concentration. Annealing reactions were analyzed by 4% PAGE in TBE buffer.

nealing was observed rapidly, within 0.5 min, and peaked after 10 min (Fig. 5A and B, lanes g to k). Low levels of spontaneous strand annealing was observed in the control alone (Fig. 5A and B, lanes b to f). The Dmc1 reaction was optimal at 5 mM $Mg(CH_3COO)_2$ (Fig. 5C, lane l). The renatured products were in the form of linear duplex as opposed to high-molecular-weight networks. Rad51 and Dmc1-dependent annealing was only slightly reduced in the absence of ATP, which suggests that renaturation by these proteins is mainly an ATP-independent process (data not shown). The strand annealing activity of budding yeast Dmc1 is also not substantially dependent on the presence of a nucleotide cofactor (26).

We then examined the ability of purified Rad51 and Dmc1 to promote strand exchange in vitro by using a circular ssDNA and homologous linear duplex DNA. Long substrates were used instead of oligonucleotides in order to increase specificity. Rad51 promoted strand exchange, with an optimal concentration of 1 monomer per 2.5 nucleotides (Fig. 6A, lane g). This is in good agreement with the ratios of one monomer of RecA or Rad51 per three nucleotides. The reaction required ATP hydrolysis, the presence of magnesium and homologous ssDNA (Fig. 6B, lanes c to f). At a ratio of 1 monomer per three nucleotides, Dmc1 was unable to perform strand exchange (Fig. 6C, lane b). However, increasing the concentration of Dmc1 to one monomer per nucleotide led to efficient strand exchange (lane i). The reaction was highly stimulated by the presence of SSB (lanes k to r). The presence of an ATP-regeneration system stimulated Dmc1-strand exchange, but ATP alone was able to support strand exchange (Fig. 6D, compare lanes b and c). Dmc1 reaction had the same cofactor requirements as Rad51 (lanes d to g), and in both cases quantification of the products revealed that the vast majority of the products were nicked circles. These results show that both Rad51 and Dmc1 are efficient recombinases in vitro.

Nucleoprotein filaments formed by Rad51 and Dmc1. Since Rad51 and Dmc1 were able to promote classical strand exchange assays, we decided to investigate whether they could form nucleoprotein filaments by electron microscopy (Fig. 7). The primary function of a nucleoprotein filament is to bring two DNA molecules into close proximity within a single filament, such that the DNA sequences are aligned ready for strand exchange and the formation of heteroduplex DNA (62).

Since meiotic recombination is thought to be initiated by DNA that contains single-stranded tails, the possibility that Rad51 may bind specifically to tailed linear duplex molecules was investigated. Rad51 bound preferentially to the ssDNA tail

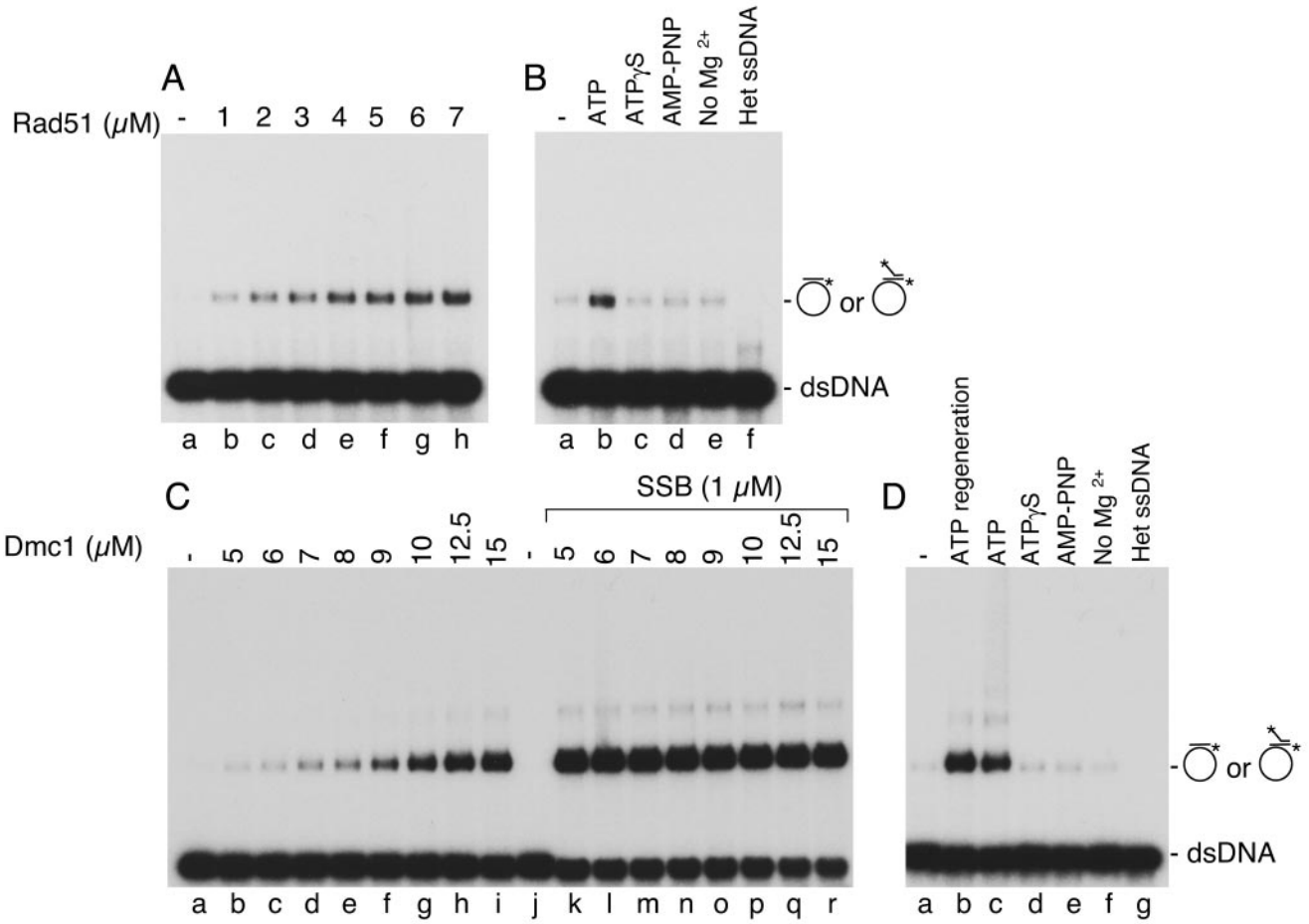

FIG. 6. Strand exchange catalyzed by *S. pombe* Rad51 (A) and Dmc1 (B). (A) DNA strand exchange as a function of Rad51 concentration (lanes a to h). (B) Cofactor dependence of Rad51-mediated strand exchange. Reactions were carried out in standard buffer with 6 μM Rad51 (lane b) or in buffer in which ATP was replaced with ATPγS (lane c) or AMP-PNP (lane d). Lane e, standard buffer lacking Mg^{2+}; lane f, pPB4.3 ssDNA was replaced with heterologous ϕX174 ssDNA; lane a, without protein. (C) Strand exchange promoted by Dmc1 (lanes a to i) and effect of SSB on Dmc1 strand exchange (lanes j to r). (D) Effect of cofactors on Dmc1 strand exchange. Reactions were carried out in buffer containing an ATP regeneration system with 12.5 μM Dmc1 (lane b), in standard buffer (lane c), or in standard buffer in which ATP was replaced with ATPγS (lane d) or AMP-PNP (lane e). Lane f, standard buffer lacking Mg^{2+}; lane g, pPB4.3 ssDNA was replaced with heterologous X174 ssDNA; lane a, without protein.

(white arrow), so that the tail was covered, whereas the duplex DNA remained essentially protein-free (black arrow) (Fig. 7A). Rad51 formed helical filaments on ssDNA (Fig. 7C). The filaments were helical and very similar in structure to the filaments formed by human Rad51 (7). In the absence of DNA, Rad51 and Dmc1 rings could be discerned (Fig. 7B and E, respectively), corroborating self-associations seen by gel filtration and two-hybrid analysis (Fig. 1C and D and 2). Consistent with a specific interaction with ssDNA, Dmc1 selectively bound the ssDNA regions of linear DNA molecules with ssDNA at both ends (Fig. 7D). Short helical filaments could be discerned (white arrows), as well as longer helical filaments (Fig. 7F). Helical nucleoprotein filaments were not observed in the absence of ATP (data not shown). Also apparent were short nucleoprotein filaments consisting of stacked rings distributed along the ssDNA (black arrow) (Fig. 7G). Regions irregular in structure could also be distinguished. The nucleoprotein filaments formed by the stacking of Dmc1 rings were very similar in structure from the filaments made by human Dmc1 (Fig. 7H). These studies are consistent with specific roles for Rad51 and Dmc1 in strand invasion during meiotic homologous recombination.

DISCUSSION

Despite the fact that the current model of recombination was proposed about 20 years ago (27), its biochemical complexity is not fully understood in mitotic cells. This statement applies even more strongly to meiotic recombination, for which the role of many proteins remains to be unraveled. Moreover, biochemical studies of human meiotic recombination have been hampered by the unavailability of human ovaries or testis and also the absence of a meiotic human cell line that can be used as a model for meiotic recombination. The characterization of Rad51 and Dmc1 in a simpler organism such as fission yeast now opens the door to biochemical studies of meiosis-specific aspects of homologous recombination. Since its discovery, Dmc1 has been functionally related to Rad51 and RecA,

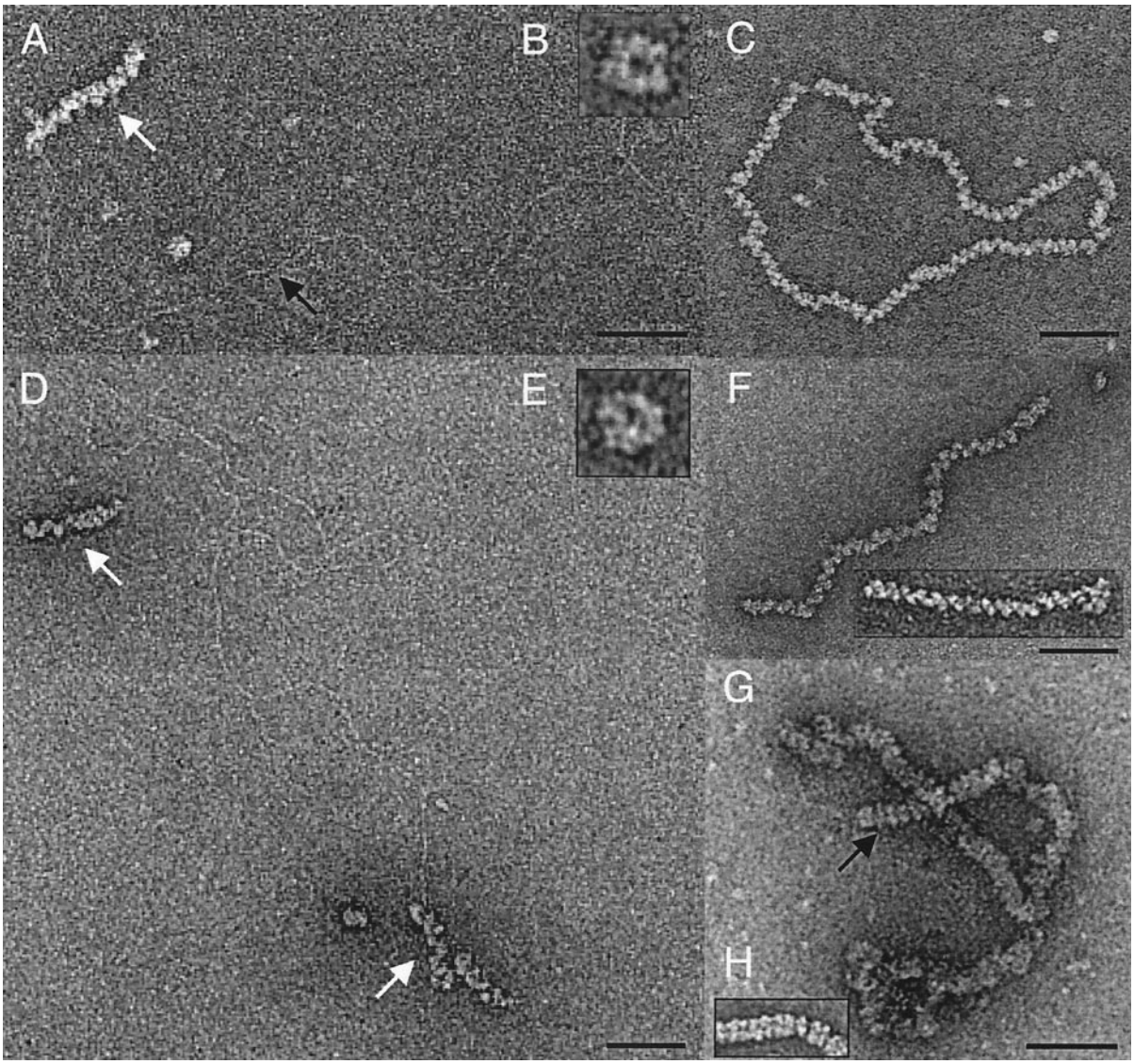

FIG. 7. Electron microscopic visualization of fission yeast Rad51 and Dmc1. (A) Electron micrograph indicating the binding of Rad51 (0.6 μM) to a linear duplex (5 μM) containing a single-stranded tail. The bound ssDNA tail is indicated by the white arrow, while the black arrow indicates unbound dsDNA. (B) Enlargement of a typical Rad51 ring. (C) Electron microscopic visualization of Rad51 (1.65 μM) bound to ϕX174 ssDNA (5 μM). (D) Electron micrograph of Dmc1 (2 μM) bound to a linear duplex with a single-stranded tail at both ends (5 μM). The white arrows indicate two short helical regions on ssDNA. (E) Magnification of a characteristic Dmc1 ring. (F) Longer helical filaments formed by Dmc1 (2 μM) on tailed DNA (5 μM) and single-strand DNA (insert). (G) Electron microscopic visualization of Dmc1 (10 μM) bound to pPB4.3 ssDNA (5 μM). A region of stacked rings is indicated by the black arrow. (H) Close-up view of a human Dmc1-dsDNA complex consisting of a series of stacked rings as a comparison. The magnification bars represent 50 nm.

the prototypical recombinase. In particular, genetic studies in budding yeast and mice have clearly established that Dmc1 is important for meiotic recombination. On the basis of these observations and sequence homology, it seemed very likely that Dmc1 protein would have properties almost identical to those of RecA and eukaryotic Rad51 proteins. The purification of *S. cerevisiae* and human Dmc1 proteins revealed that the Dmc1 family had both similar and distinct features. Hence, the present study was begun to investigate the limited recombinase activity found in Dmc1, as well as its unusual structural properties on DNA for a member of the RecA/Rad51 family of enzymes.

Formation of multimeric rings by Dmc1. A remarkable feature of many enzymes involved in DNA recombination is their ability to self-interact to form multimeric rings (25). Gel filtration analysis revealed that Rad51 and Dmc1 form homo-oligomers. Electron microscopic visualization of human Dmc1 revealed that it forms octameric ring structures, with a diameter of ca. 11 to 12 nm and a central cavity of 2 to 4 nm (34). Electron micrographs of *S. pombe* Rad51 and Dmc1 rings revealed structures of the same dimensions. When human and *S. pombe* Dmc1 were compared by electron microscopy, almost all purified human Dmc1 was seen as rings in the absence of DNA, whereas *S. pombe* Dmc1 formed rings but also a pro-

portion of the protein was seen as incomplete rings and C-shaped rings (data not shown). This was also apparent in gel filtration experiments in which Dmc1 eluted in a broad profile, suggesting that it can form structures containing a variable number of molecules. These observations may be due to the weaker self-interactions between the pombe Dmc1 monomer, allowing the protein to dissociate. It might be particularly important in nucleoprotein filament formation since each ring must dissociate into protein monomers that will load on DNA.

Since Dmc1 and Rad51 form rings in solution and also helices on DNA it was conceivable that the proteins would self-interact. Domain mapping experiments by two-hybrid analysis revealed that the self-interaction domain of Dmc1 was present at the N terminus of the protein. The N-terminal domain of human Dmc1 is also predicted to be located near the monomer-monomer interface and as been suggested to have a role in ring stability (31). In support of this, partial degradation of hDmc1 at the N terminus led to heptameric rings (41). Similarly, the N-terminal region of budding yeast Rad51 also mediates self-interaction (13).

Genetic studies have established that both Dmc1 and Rad51 not only can promote meiotic recombination in the absence of one or the other but also can cooperate during a large fraction of recombination events. Dmc1 and Rad51 colocalize to a high extent at the time when meiotic recombination occurs in *S. cerevisiae*, plants, and mammals (1, 8, 56). Although Rad51 and Dmc1 did not interact in two-hybrid assays, this might not be the best way to look at heterotypic interactions between Rad51 and Dmc1 since heterologous interactions may be masked by the strong self-interaction of both proteins. In fact, the proteins are most likely to interact as their active form on DNA-helical filaments. This might be a difficult experiment to do since both proteins bind DNA.

Conserved features of Rad51 and Dmc1. As expected for a member of the RecA family, Rad51 and Dmc1 can hydrolyze ATP. However, it is now clear that eukaryotic homologs of RecA, including budding yeast and human Rad51, have a much lower ATP hydrolysis activity. For instance, the k_{cat} of RecA is estimated to be 30 min^{-1}, whereas human Rad51 and *S. pombe* Rad51 and Dmc1 (the present study) have estimated hydrolysis rates at 0.16, 0.7, and 0.6 min^{-1}, respectively (4). Nevertheless, despite the less efficient ATPase activity of these proteins, low ATP hydrolysis may be still relevant in vivo. The mouse embryonic stem cell line expressing human RAD51-K133R, a form competent for ATP binding but defective for ATP hydrolysis, shows hypersensitivity to mitomycin C, ionizing radiation, and a decrease in sister chromatid exchange (53). Similarly, budding yeast *DMC1-G126D* dominant allele mutated in the first ATP binding motif confer a null phenotype (17).

Another conserved feature of Rad51 and Dmc1 was that both proteins bound ssDNA preferentially over dsDNA. Preferential binding to ssDNA occurred on a variety of substrates, excluding the possibility of sequence-specific preferences in our assays (data not shown). The preferential interaction with ssDNA tails may have important biological consequences at the initiation of recombination during meiosis since DSBs are processed into 3′-tailed DNA molecules.

Strand annealing and DNA strand exchange by Dmc1. Renaturation of complementary ssDNAs has been reported for various proteins involved in DNA metabolism, including RecA (61), human Rad51 (21), and yeast and human Rad52 (38, 59). The activity observed for Rad51 and Dmc1 may be relevant since a fraction of meiotic DSBs is repaired by synthesis-dependent strand annealing (40). In this model, following the initial strand invasion and repair synthesis, the invading strand containing newly synthesized DNA is displaced and reannealed to the other 3′ end.

Using single-stranded circular DNA and linear duplex substrates, Rad51- and Dmc1-mediated strand exchange was observed. In vivo data also support the notion that Dmc1 is involved in strand invasion, since yeast mutants defective in *DMC1* accumulate abnormally high levels of DSBs and exhibit hyper-resection at these ends (9). Before 2004, previous studies have shown that with human Dmc1, strand exchange was observed only with oligonucleotides or nicked-duplex DNA but not in the presence of longer substrates (22, 32, 34). Our results are in accordance with recent studies showing that human Dmc1 also forms nucleoprotein filaments and promotes efficient strand exchange (48). Unlike human Dmc1, fission yeast Dmc1 was able to promote strand exchange in the absence of a single-strand binding protein. Apart from the fact that we are comparing protein from different species, this difference is perhaps due the use of smaller duplex DNA in our assays. Like human Dmc1 with human RPA (48), strand exchange by Dmc1 is highly stimulated by a ssDNA binding protein such as SSB. The functional analogy between RPA and SSB is supported by results indicating that SSB can substitute for budding yeast and human RPA in strand exchange mediated by *S. cerevisiae* and human Rad51, respectively (5, 55). Since Dmc1 has the tendency to aggregate ssDNA, the purpose of SSB might be to minimize secondary structures in the ssDNA. Moreover, it could also sequester the displaced strand resulting from strand exchange (48). In addition to studies on the human Dmc1 (48), our data suggest that Dmc1 is an efficient recombinase with very similar structural properties to Rad51 in evolutionary divergent species highlighting the universality of its mode of action.

Although *S. pombe* Dmc1 can form homologous contacts in vitro, the efficiency of these reactions is low compared to RecA. This suggests that other factors may be required to increase the efficiency of Dmc1-mediated strand exchange. Human Dmc1 D-loop formation has been found to be stimulated by the human RAD54B protein (48). Budding yeast Hop2-Mnd1 and mouse Hop2 can also stimulate Dmc1 specifically (14, 18). Hence, it is very likely that fission yeast proteins will also stimulate Dmc1. Nonprotein cofactors such as Ca^{2+} might also stimulate Dmc1. This is of particular interest since Ca^{2+} stimulates DNA strand exchange of human Rad51 by modulating its ATPase activity. A dependence of meiosis on Ca^{2+} has been observed during meiosis I, when homologous recombination occurs (10).

Rings or nucleoproteins filaments by Dmc1? It is well known that helical nucleoprotein filaments represent the biologically active form of RecA/Rad51. However, it was reported in 1999 that human Dmc1 formed stacked octameric rings on DNA (34, 41). The nonhelical filaments appeared more similar to those made by *E. coli* RecT protein than to those made by RecA (57). In the present study, we show for the first time that a member of the Dmc1 family from lower eukaryotes is able to

form helical nucleoprotein filaments on DNA, as well as stacked rings. This is not the first example since RadA protein also exists in two oligomeric states that can bind DNA: an octameric ring and a helical filament (36, 63). The helical filaments of Dmc1 are most likely responsible for the strand exchange activity and represent the catalytic intermediate of this reaction. ssDNA enclosed within Dmc1 rings would not be able to continuously synapse with the homologous duplex DNA. We suppose that helical filaments are not as stable as Dmc1 stacked rings, and this is why stacked rings are more easily detectable. This may also explain why a higher concentration of Dmc1 is required for optimal strand exchange. Certainly, a major unresolved issue is to find the exact functions of the Dmc1 stacked rings. These structures are likely important since they are characteristic of a meiosis-specific protein originating from *S. pombe*. This organism displays a very simple meiosis with potentially the most primitive features of meiotic mechanisms (52). The evolutionary conservation of this feature should be significant perhaps at the chromosomal level rather than with naked DNA substrates.

Besides a role in facilitating meiotic recombination, it has been proposed that the main function of Dmc1 is to pair homologous chromosomes during meiosis. In support of this, synaptonemal complex formation and pairing of homologous chromosomes is independent of meiotic recombination in organisms lacking Dmc1, such as fruit fly and nematode. Importantly, *S. pombe* cells lack the synaptonemal complex but possess structures called linear elements that appear to be analogous to the axial elements of the synaptonemal complex (52). It was not clear how Dmc1 would participate in chromosome pairing and recombination if it was a weak recombinase in vitro. Our results add significant strength to the idea that the recombinase function of Dmc1 mediated by helical nucleoprotein filaments is crucial for the synapsis and proper segregation of homologous chromosomes as well as genetic diversity. These functions might have profound biological consequences for human fertility.

ACKNOWLEDGMENTS

We are grateful to Isabelle Brodeur, Steve West, and Rhea Utley for helpful comments on the manuscript. We also thank Steve West and Jacques Dubochet for support and interest, Alexander Mazin for the communication of unpublished data, and Hiroyuki Yamano for the gift of *S. pombe* cDNA and genomic libraries.

A.S. is supported by Swiss National Foundation grant 3100A0-103962. J.-Y.M. is a Canadian Institutes of Health New Investigator, and this research is supported by funds from the National Cancer Institute of Canada and the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

1. **Anderson, L. K., H. H. Offenberg, W. M. H. C. Verkuijlen, and C. Heyting.** 1997. RecA-like proteins are components of early meiotic nodules in lily. Proc. Natl. Acad. Sci. USA **94:**6868–6873.
2. **Baudat, F., K. Manova, J. P. Yuen, M. Jasin, and S. Keeney.** 2000. Chromosome synapsis defects and sexually dimorphic meiotic progression in mice lacking Spo11. Mol. Cell **6:**989–998.
3. **Baumann, P., F. E. Benson, N. Hajibagheri, and S. C. West.** 1997. Purification of human Rad51 protein by selective spermidine precipitation. Mutat. Res. DNA Repair **384:**65–72.
4. **Baumann, P., F. E. Benson, and S. C. West.** 1996. Human Rad51 protein promotes ATP-dependent homologous pairing and strand transfer reactions in vitro. Cell **87:**757–766.
5. **Baumann, P., and S. C. West.** 1999. Heteroduplex formation by human Rad51 protein: effects of DNA end-structure, hRP-A and hRad52. J. Mol. Biol. **291:**363–374.
6. **Baumann, P., and S. C. West.** 1997. The human Rad51 protein: polarity of strand transfer and stimulation by hRP-A. EMBO J. **16:**5198–5206.
7. **Benson, F. E., A. Stasiak, and S. C. West.** 1994. Purification and characterisation of the human Rad51 protein, an analogue of *Escherichia coli* RecA. EMBO J. **13:**5764–5771.
8. **Bishop, D. K.** 1994. RecA homologs Dmc1 and Rad51 interact to form multiple nuclear complexes prior to meiotic chromosome synapsis. Cell **79:**1081–1092.
9. **Bishop, D. K., D. Park, L. Z. Xu, and N. Kleckner.** 1992. DMC1: a meiosis-specific yeast homolog of *Escherichia coli* RecA required for recombination, synaptonemal complex formation, and cell cycle progression. Cell **69:**439–456.
10. **Bugreev, D. V., and A. V. Mazin.** 2004. Ca^{2+} activates human homologous recombination protein Rad51 by modulating its ATPase activity. Proc. Natl. Acad. Sci. USA **101:**9988–9993.
11. **Catlett, M. G., and S. L. Forsburg.** 2003. *Schizosaccharomyces pombe* Rdh54 (TID1) acts with Rhp54 (RAD54) to repair meiotic double-strand breaks. Mol. Biol. Cell **14:**4707–4720.
12. **Cervantes, M. D., J. A. Farah, and G. R. Smith.** 2000. Meiotic DNA breaks associated with recombination in *Schizosaccharomyces pombe*. Mol. Cell **5:**883–888.
13. **Chen, J. J., D. P. Silver, D. Walpita, S. B. Cantor, A. F. Gazdar, G. Tomlinson, F. J. Couch, B. L. Weber, T. Ashley, D. M. Livingston, and R. Scully.** 1998. Stable interaction between the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells. Mol. Cell **2:**317–328.
14. **Chen, Y. K., C. H. Leng, H. Olivares, M. H. Lee, Y. C. Chang, W. M. Kung, S. C. Ti, Y. H. Lo, A. H. Wang, C. S. Chang, D. K. Bishop, Y. P. Hsueh, and T. F. Wang.** 2004. Heterodimeric complexes of Hop2 and Mnd1 function with Dmc1 to promote meiotic homolog juxtaposition and strand assimilation. Proc. Natl. Acad. Sci. USA **101:**10572–10577.
15. **Davies, A. A., J.-Y. Masson, M. J. McIlwraith, A. Z. Stasiak, A. Stasiak, A. R. Venkitaraman, and S. C. West.** 2001. Role of BRCA2 in control of the RAD51 recombination and DNA repair protein. Mol. Cell **7:**273–282.
16. **Davis, L., and G. R. Smith.** 2001. Meiotic recombination and chromosome segregation in Schizosaccharomyces pombe. Proc. Natl. Acad. Sci. USA **98:**8395–8402.
17. **Dresser, M. E., D. J. Ewing, M. N. Conrad, A. M. Dominguez, R. Barstead, H. Jiang, and T. Kodadek.** 1997. Dmc1 functions in a *Saccharomyces cerevisiae* meiotic pathway that is largely independent of the Rad51 pathway. Genetics **147:**533–544.
18. **Enomoto, R., T. Kinebuchi, M. Sato, H. Yagi, T. Shibata, H. Kurumizaka, and S. Yokoyama.** 2004. Positive role of the mammalian TBPIP/HOP2 protein in DMC1-mediated homologous pairing. J. Biol. Chem. **279:**35263–35272.
19. **Fukushima, K., Y. Tanaka, K. Nabeshima, T. Yoneki, T. Tougan, S. Tanaka, and H. Nojima.** 2000. Dmc1 of *Schizosaccharomyces pombe* plays a role in meiotic recombination. Nucleic Acids Res. **28:**2709–2716.
20. **Grishchuk, A. L., R. Kraehenbuehl, M. Molnar, O. Fleck, and J. Kohli.** 2003. Genetic and cytological characterization of the RecA-homologous proteins Rad51 and Dmc1 of *Schizosaccharomyces pombe*. Curr. Genet. **165:**1031–1043.
21. **Gupta, R. C., L. R. Bazemore, E. I. Golub, and C. M. Radding.** 1997. Activities of human recombination protein Rad51. Proc. Natl. Acad. Sci. USA **94:**463–468.
22. **Gupta, R. C., E. Golub, B. Bi, and C. M. Radding.** 2001. The synaptic activity of HsDmc1, a human recombination protein specific to meiosis. Proc. Natl. Acad. Sci. USA **98:**8433–8439.
23. **Habu, T., T. Taki, A. West, Y. Nishimune, and T. Morita.** 1996. The mouse and human homologs of DMC1, the yeast meiosis-specific homologous recombination gene, have a common unique form of exon skipped transcript in meiosis. Nucleic Acids Res. **24:**470–477.
24. **Habu, T., N. Wakabayashi, K. Yoshida, K. Yomogida, Y. Nishimune, and T. Morita.** 2004. p53 protein interacts specifically with the meiosis-specific mammalian RecA-like protein DMC1 in meiosis. Carcinogenesis **25:**889–893.
25. **Hingorani, M. M., and M. O'Donnell.** 1998. Toroidal proteins: running rings around DNA. Curr. Biol. **8:**R83–R86.
26. **Hong, E. R. L., A. Shinohara, and D. K. Bishop.** 2001. *Saccharomyces cerevisiae* Dmc1 protein promotes renaturation of single-strand DNA (ssDNA) and assimilation of ssDNA into homologous supercoiled duplex DNA. J. Biol. Chem. **276:**41906–41912.
27. **Howard-Flanders, P., S. C. West, and A. J. Stasiak.** 1984. Role of RecA spiral filaments in genetic recombination. Nature **309:**215–220.
28. **Hunter, N., and N. Kleckner.** 2001. The single-end invasion: an asymmetric intermediate at the double-strand break to double-Holliday junction transition of meiotic recombination. Cell **106:**59–70.
29. **James, P., J. Halladay, and E. A. Craig.** 1996. Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. Genetics **144:**1425–1436.
30. **Keeney, S.** 2001. Mechanism and control of meiotic recombination initiation. Curr. Top. Dev. Biol. **52:**1–53.
31. **Kinebuchi, T., W. Kagawa, R. Enomoto, K. Tanaka, K. Miyagawa, T. Shi-**

bata, H. Kurumizaka, and S. Yokoyama. 2004. Structural basis for octameric ring formation and DNA interaction of the human homologous-pairing protein Dmc1. Mol. Cell **14:**363–374.
32. **Li, Z. F., E. I. Golub, R. Gupta, and C. M. Radding.** 1997. Recombination activities of HsDmc1 protein, the meiotic human homolog of RecA protein. Proc. Natl. Acad. Sci. USA **94:**11221–11226.
33. **Lim, D. S., and P. Hasty.** 1996. A mutation in mouse RAD51 results in an early embryonic lethal that is suppressed by a mutation in p53. Mol. Cell. Biol. **16:**7133–7143.
34. **Masson, J.-Y., A. A. Davies, N. Hajibagheri, E. Van Dyck, F. E. Benson, A. Z. Stasiak, A. Stasiak, and S. C. West.** 1999. The meiosis-specific recombinase hDmc1 forms rings structures and interacts with hRad51. EMBO J. **18:**6552–6560.
35. **Masson, J.-Y., and S. C. West.** 2001. The Rad51 and Dmc1 recombinases: a non-identical twin relationship. Trends Biochem. Sci. **26:**131–136.
36. **McIlwraith, M. J., D. R. Hall, A. Z. Stasiak, A. Stasiak, D. B. Wigley, and S. C. West.** 2001. RadA protein from *Archeoglobus fulgidans* forms rings, nucleoprotein filaments and catalyzes homologous recombination. Nucleic Acids Res. **29:**4509–4517.
37. **McIlwraith, M. J., E. Van Dyck, J.-Y. Masson, A. Z. Stasiak, A. Stasiak, and S. C. West.** 2000. Reconstitution of the strand invasion step of double-strand break repair using human Rad51, Rad52 and RPA proteins. J. Mol. Biol. **304:**151–164.
38. **Mortensen, U. H., C. Bendixen, I. Sunjevaric, and R. Rothstein.** 1996. DNA strand annealing is promoted by the yeast Rad52 protein. Proc. Natl. Acad. Sci. USA **93:**10729–10734.
39. **Neyton, S., F. Lespinasse, P. B. Moens, R. Paul, P. Gaudray, V. Paquis-Flucklinger, and S. Santucci-Darmanin.** 2004. Association between MSH4 (MutS homologue 4) and the DNA strand-exchange RAD51 and DMC1 proteins during mammalian meiosis. Mol. Hum. Reprod. **10:**917–924.
40. **Paques, F., and J. E. Haber.** 1999. Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. **63:**349–404.
41. **Passy, S. I., X. Yu, Z. Li, C. M. Radding, J.-Y. Masson, S. C. West, and E. H. Egelman.** 1999. Human Dmc1 protein binds DNA as an octameric ring. Proc. Natl. Acad. Sci. USA **96:**10684–10688.
42. **Petersen, G., and O. Seberg.** 2002. Molecular evolution and phylogenetic application of DMC1. Mol. Phylogenet. Evol. **22:**43–50.
43. **Pittman, D. L., J. Cobb, K. J. Schimenti, L. A. Wilson, D. M. Cooper, E. Brignull, M. A. Handel, and J. C. Schimenti.** 1998. Meiotic prophase arrest with failure of chromosome synapsis in mice deficient for Dmc1, a germline-specific recA homolog. Mol. Cell **1:**697–705.
44. **Pugh, B. F., and M. M. Cox.** 1987. Stable binding of RecA protein to duplex DNA. J. Biol. Chem. **262:**1326–1336.
45. **Rockmill, B., M. Sym, H. Scherthan, and G. S. Roeder.** 1995. Roles for two RecA homologs in promoting meiotic chromosome synapsis. Genes Dev. **9:**2684–2695.
46. **Romanienko, P. J., and R. D. Camerini-Otero.** 2000. The mouse Spo11 gene is required for meiotic chromosome synapsis. Mol. Cell **6:**975–987.
47. **Schwacha, A., and N. Kleckner.** 1997. Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog only pathway. Cell **90:**1123–1135.
48. **Sehorn, M. G., S. Sigurdsson, W. Bussen, V. M. Unger, and P. Sung.** 2004. Human meiotic recombinase Dmc1 promotes ATP-dependent homologous DNA strand exchange. Nature **429:**433–437.
49. **Siaud, N., E. Dray, I. Gy, E. Gerard, N. Takvorian, and M. P. Doutriaux.** 2004. Brca2 is involved in meiosis in *Arabidopsis thaliana* as suggested by its interaction with Dmc1. EMBO J. **23:**1392–1401.
50. **Smith, K. N., and A. Nicolas.** 1998. Recombination at work for meiosis. Curr. Opin. Genet. Dev. **8:**200–211.
51. **Sogo, J., A. Stasiak, W. De Bernadin, R. Losa, and T. Koller.** 1987. Binding of proteins to nucleic acids as studied by electron microscopy, p. 61–79. *In* J. Sommerville and U. Scheer (ed.), Electron microscopy in molecular biology. IRL Press, Oxford, England.
52. **Solari, A. J.** 2002. Primitive forms of meiosis: the possible evolution of meiosis. Biocell **26:**1–13.
53. **Stark, J. M., P. Hu, A. J. Pierce, M. E. Moynahan, N. Ellis, and M. Jasin.** 2002. ATP hydrolysis by mammalian RAD51 has a key role during homology-directed DNA repair. J. Biol. Chem. **277:**20185–20194.
54. **Story, R. M., I. T. Weber, and T. A. Steitz.** 1992. The structure of the *Escherichia coli* RecA protein monomer and polymer. Nature **355:**318–325.
55. **Sugiyama, T., E. M. Zaitseva, and S. C. Kowalczykowski.** 1997. A single-stranded DNA-binding protein is needed for efficient complex formation by the *Saccharomyces cerevisiae* Rad51 protein. J. Biol. Chem. **272:**7940–7945.
56. **Tarsounas, M., T. Morita, R. E. Pearlman, and P. B. Moens.** 1999. RAD51 and DMC1 form mixed complexes associated with mouse meiotic chromosome cores and synaptonemal complexes. J. Cell Biol. **147:**207–219.
57. **Thresher, R. J., A. M. Makhov, S. D. Hall, R. Kolodner, and J. D. Griffith.** 1995. Electron microscopic visualization of RecT protein and its complexes with DNA. J. Mol. Biol. **254:**364–371.
58. **Tsuzuki, T., Y. Fujii, K. Sakuma, Y. Tominaga, K. Nakao, M. Sekiguchi, A. Matsushiro, Y. Yoshimura, and T. Morita.** 1996. Targeted disruption of the RAD51 gene leads to lethality in embryonic mice. Proc. Natl. Acad. Sci. USA **93:**6236–6240.
59. **Van Dyck, E., A. Z. Stasiak, A. Stasiak, and S. C. West.** 2001. Visualization of recombination intermediates produced by RAD52-mediated single-strand annealing. EMBO J. **2:**905–909.
60. **Wang, W., and B. A. Malcolm.** 1999. Two-stage PCR protocol allowing introduction of multiple mutations, deletions and insertions using QuikChange site-directed mutagenesis. BioTechniques **26:**680–682.
61. **Weinstock, G. M., K. McEntee, and I. R. Lehman.** 1979. ATP-dependent renaturation of DNA catalyzed by the RecA protein of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **76:**126–130.
62. **West, S. C.** 1992. Enzymes and molecular mechanisms of homologous recombination. Annu. Rev. Biochem. **61:**603–640.
63. **Yang, S., X. Yu, E. M. Seitz, S. C. Kowalczykowski, and E. H. Egelman.** 2001. Archaeal RadA protein binds DNA as both helical filaments and octameric rings. J. Mol. Biol. **314:**1077–1085.
64. **Yoshida, K., G. Kondoh, Y. Matsuda, T. Habu, Y. Nishimune, and T. Morita.** 1998. The mouse *recA*-like gene DMC1 is required for homologous chromosome synapsis during meiosis. Mol. Cell **1:**707–718.