Homologous recombination, which is critical to genetic diversity, depends on homologous pairing (HP). HP is the switch from parental to recombinant base pairs, which requires expansion of inter-base pair spaces. This expansion unavoidably causes untwisting of the parental double-stranded DNA. RecA/Rad51-catalyzed ATP-dependent HP is extensively stimulated in vitro by negative supercoils, which compensates for untwisting. However, in vivo, double-stranded DNA is relaxed by bound proteins and thus is an unfavorable substrate for RecA/Rad51. In contrast, Mhr1, an ATP-independent HP protein required for yeast mitochondrial homologous recombination, catalyzes HP without the net untwisting of double-stranded DNA. Therefore, we questioned whether Mhr1 uses a novel strategy to promote HP. Here, we found that, like RecA, Mhr1 induced the extension of bound single-stranded DNA. In addition, this structure was induced by all evolutionarily and structurally distinct HP proteins so far tested, including bacterial RecO, viral RecT, and human Rad51. Thus, HP includes the common non-canonical DNA structure and uses a common core mechanism, independent of the species of HP proteins. We discuss the significance of multiple types of HP proteins.

Homologous recombination (HR) is essential for gametogenesis during meiosis and plays an important role in the generation of genetic diversity, a process that is critical for natural selection. A general HR intermediate is the heteroduplex joint, which is formed between a single-stranded (ss) DNA tail derived from a double-stranded break and a homologous double-stranded (ds) DNA by homologous pairing (HP) and subsequent strand exchange. HP is a switch from parental dsDNA base pairs to recombinant base pairs involving the ssDNA and the complementary strand of the dsDNA, which form the core of the recombination intermediate, and strand exchange is the unidirectional replacement of a dsDNA strand by the incoming ssDNA. The RecA/Rad51 family of proteins, which include bacterial RecA, archaeal RadA/Rad51, eukaryotic Rad51, and meiosis-specific Dmc1, are essential for HR in their respective organisms, and these proteins can promote ATP-dependent HP and ATP hydrolysis-dependent strand exchange in vitro (see Refs. 5–10 for reviews). In HP, ATP-bound RecA first binds to ssDNA, and this ssDNA-RecA complex then interacts with dsDNA without homologous recognition. Within the RecA-ssDNA-dsDNA complex, a homologous region is identified (11). The base pair switch in HP is formally carried out by base rotation or base flipping (rotation around the base-sugar bond), either of which requires the expansion of the spaces between neighboring bases or base pairs (see Refs. 7 and 12).

Electron microscopic studies have shown that RecA/Rad51 proteins form a well conserved right-handed helical filament around ssDNA or dsDNA (13–15). In the absence of ATP, these proteins assemble as a shorter inactive filament (helical pitch, 65–85 Å) (16, 17). In the presence of ATP (or ATPγS, a non-hydrolyzable ATP analogue), the filament adopts an extended active conformation with a helical pitch of ~95 Å, and the contour length of ssDNA and dsDNA within the active filament is elongated to the same extent (13–15, 17, 18). This equalized elongation has been inferred to widen the spacing between bases of ssDNA and dsDNA equally in the nucleoprotein filament to facilitate the homologous alignment of both DNA substrates to achieve base pair switching (13). Previously, we analyzed the three-dimensional structure of the RecA-ssDNA complex in the presence of ATPγS by NMR, which showed that the axial rise per ssDNA base was extended to nearly 5 Å (19), and that the interconversion of sugar pucker induced horizontal base rotation (20). On the basis of these results, and as there was no other structural information at that time, we proposed a base rotation mechanism to explain the base pair switch in HP by assuming that the ssDNA and dsDNA were extended equally and uniformly (20). Recently, the crystal structure of the RecA-ssDNA complex has revealed a non-uniformly extended structure for the ssDNA (21). The crystal...
structure contains “a three-nucleotide segment” (triplet) region and “a long untwisted inter-nucleotide” (inter-triplet) region (see Fig. 4). However, it remains unclear which structure contributes to HP and how it does so.

The extension of dsDNA is unavoidably associated with untwisting of the double helix (18, 20). Actually, the dsDNA was shown to be untwisted (unwound) within the homology-independent RecA-ssDNA-dsDNA intermediate of HP described above (22, 23). HP mediated by RecA or Rad51 was shown to be extensively stimulated by negative supercoiling of the dsDNA substrate in vitro (24, 25). This is probably because negative supercoils in the dsDNA substrate would compensate for the positive supercoils generated by the untwisting for HP. Closed circular dsDNA isolated from living cells, including DNA from bacteria, nuclei, or mitochondria of eukaryotic cells, is similarly supercoiled. However, in vivo, the supercoils of cellular dsDNA are relaxed by nucleosome assembly in eukaryotic nuclei and by the binding of HU (26) and/or other DNA-binding proteins in bacteria. Thus, dsDNA in vivo is an unfavorable substrate for HP mediated by RecA/Rad51 family proteins.

In mitochondria, which do not have RecA/Rad51 family proteins, negative supercoils are relaxed by the binding of TFAM (in mammals) or Abf2 (in yeast) (27, 28). In this in vivo dsDNA state, Mhr1, an ATP-independent HP protein required for mitochondrial HR in the budding yeast Saccharomyces cerevisiae (29) catalyzes HP without the net untwisting of dsDNA, i.e. Mhr1 catalyzes HP with relaxed closed circular dsDNA with similar efficiency as with dsDNA lacking topological constraints (linear dsDNA and closed circular dsDNA in the presence of a topoisomerase) (30). Furthermore, in contrast to what is observed for RecA/Rad51, Mhr1-catalyzed HP is prevented by negative dsDNA supercoiling. The absence of net untwisting of dsDNA appears at first glance to mean HP without the untwisting of the parental dsDNA. However, HP requires the expansion of inter-base pair spaces for base pair switching as described above, and thus we proposed that right-handed wrapping of dsDNA around Mhr1 with an extended and untwisted configuration allows base rotation for HP (30). However, it remained to be experimentally determined whether Mhr1 and RecA/Rad51 share a common or different mechanism for HP.

In addition to Mhr1, several proteins that promote HP in vitro in the absence of nucleotide cofactors have been identified. These include the human (hs) Xrcc3-Rad51c/Rad51L2 complex (human Rad51 paralogues; 31), hsRad52 (32), Escherichia coli (ec) phage λ β-protein (33), ecRecT (a homologue of λ β-protein 34), ecRecO (35), and Ustilago maydis Brh2 (36). Some of these proteins are termed recombination mediators, but we refer to them as ATP-independent HP proteins for the purpose of this study (supplemental Fig. S1). In contrast to RecA/Rad51 family members, ATP-independent HP proteins, except for those in the Xrcc3-Rad51C complex, do not exhibit any amino acid sequence homology with RecA/Rad51 proteins or other ATP-independent HP proteins. In addition, ATP-independent HP proteins exhibit significantly different quaternary structures (31, 32, 36–40). The N-terminal domain of hsRad52 forms an undecameric ring around which ssDNA and/or dsDNA wrap(s) (32, 41), and the interaction of closed circular dsDNA with hsRad52 generates negative supercoils (41), whereas binding to RecA generates positive supercoils in this substrate. On the other hand, like RecA, RecT was shown to untwist dsDNA during HP (42). The binding of dsDNA to Mhr1 causes neither untwisting nor twisting (30). Thus, the properties of HP proteins vary considerably except for their HP activities.

In this study, we questioned whether the extended structure of ssDNA as seen in the RecA-ssDNA complex is conserved among HP proteins, or whether each HP protein uses a different principle to promote HP. If the extended structure is a common determinant of HP, the different HP proteins are likely to use a common mechanism to promote HP, but their variation may reflect requirements for optimizing HP in different cellular environments. Thus, we focused on the structure of the HP protein-bound ssDNA, an HP intermediate. We determined the three-dimensional structures of ssDNA bound to Mhr1 and of three other evolutionarily distinct HP proteins, ecRecT (ATP-independent, from the λ-like cryptic prophage Rac of E. coli, involved in plasmid HR (43)), Thermus thermophilus (tt) RecO (ATP-independent HP protein in bacteria), and hsRad51 (ATP-dependent, human nuclear homologue of RecA) and compared them with ssDNA bound to ecRecA (E. coli, the prototype of the RecA family; supplemental Fig. S1). This is the first demonstration that diverse HP proteins, both ATP-dependent (RecA/Rad51) and ATP-independent (Mhr1, RecO, RecT), use the non-canonical extended DNA structure as a common intermediate for HP, and this suggests that they use a common mechanism for HP.

**EXPERIMENTAL PROCEDURES**

**Oligonucleotides**—d(TACG) was synthesized by Espec Oligo Service, Japan. The r(UACG) was synthesized by Hokkaido System Science, Japan.

**Protein Expression and Purification**—E. coli BL21(DE3)-pLysS cells were transformed with an expression vector, pET14b, containing the ecRecT gene. The expression of ecRecT was induced by the addition of isopropyl 1-thio-β-D-galactopyranoside for 4 h at 37 °C. Cells were harvested and suspended in an isotonic solution (25 mM Tris-Cl, pH 8.0, 50 mM glucose, 5 mM 2-mercaptoethanol, and 0.1 mM APMSF). Cells were disrupted by sonication on ice, after which 0.5 M NaCl was added to the lysate. Following centrifugation (1 h at 60,000 × g), supernatants were applied to a nickel-nitrilotriacetic acid Superflow (Qiagen, Valencia, CA) column with NAT buffer (50 mM NaPO4, pH 7.5, 150 mM NaCl, 5 mM imidazole, 10% glycerol, and 0.1 mM APMSF). After washing with NAT buffer, ecRecT was eluted with a linear imidazole gradient (100–400 mM). RecT-containing fractions were dialyzed against SAT buffer (20 mM Tris-Cl, pH 7.5, 150 mM NaCl, 10% glycerol, and 0.01 mM APMSF) and then loaded onto an SP Sepharose (GE Healthcare, UK) column. The column was washed with SAT buffer and ecRecT was eluted with a linear NaCl gradient (150–1000 mM). After removal of the His6 tag using a Thrombin Cleavage Capture Kit (Merck), purified ecRecT was concentrated by Centriprep and Centricon (Millipore, Billerica, MA).
in 20 mM Tris-Cl, pH 7.5, 100 mM NaCl, and stored on ice until use.

For the expression of recombinant Mhr1, E. coli BL21(DE3)-pLysS ΔrecA cells were transformed with the expression vector pET14b-Mhr1 (29). Cells were grown at 37 °C for 4 h and recombinant protein expression was then induced by the addition of 1 mM isopropyl 1-thio-β-D-galactopyranoside at 18 °C for 16 h. Cells were harvested, and the supernatant was prepared as described above. Following the addition of 1 mM ammonium sulfate, the supernatant was applied to a Phenyl-TOYOPEARL (TOSOH, Japan) column with PA buffer (25 mM Tris-Cl, pH 7.5, 1 mM ammonium sulfate, 5 mM 2-mercaptoethanol, and 0.01 mM APMSF). The column was washed with the same buffer, and Mhr1 was then eluted using PB buffer (25 mM Tris-Cl, pH 7.5, 5 mM 2-mercaptoethanol, 10% glycerol, and 0.01 mM APMSF). The Mhr1-containing fraction was loaded onto a nickel-nitrilotriacetic acid Superflow (Qiagen) column with NAM buffer (25 mM MES-OH, pH 7.5, 500 mM NaCl, 0.05% Triton X-100 (Sigma), 50 mM imidazole, 7% glycerol, and 0.01 mM APMSF). The column was washed with NBM buffer (25 mM Tris-Cl, pH 7.5, 500 mM NaCl, 0.05% Triton X-100 (Sigma), 50 mM imidazole, 7% glycerol, and 0.01 mM APMSF) and Mhr1 was eluted using a linear imidazole gradient (50–400 mM). Mhr1 fractions were diluted 9-fold in SA buffer (25 mM MES-OH, pH 6.5, 10% glycerol, 1 mM EDTA, 5 mM 2-mercaptoethanol, and 0.01 mM APMSF) and then loaded onto an SP Sephrose fast flow (GE Healthcare) column pre-equilibrated with SA buffer. Following cleavage of the histidine tag, Mhr1 was concentrated in 25 mM MES-OH, pH 6.5, and then stored on ice prior to use. The recombinant proteins ecRecA, ttRecO, ttSSB, and human Rad51 were purified as previously described, with some minor modifications (37, 44–46).

Solution Measurements (Transferred Nuclear Overhauser Effect (TRNOE))—NMR sample preparation and TRNOE Spectroscopy (TRNOESY) measurements were performed as previously reported (19), with some exceptions. Protein samples were concentrated and dissolved in deuterium (2H2O) NMR buffer. Undesirable organic impurities and metal ions were removed from the ssDNA oligomer d(TAGC) and the ssRNA oligomer r(UACG) using a cation-exchange resin (47). The concentrations of purified ssDNA and ssRNA were determined by absorbance at 260 nm. Protein samples, ssDNA, ssRNA, ATPγS (Roche Applied Science), and AMPPPN (Sigma) were lyophilized and dissolved in 2H2O prior to mixing. TRNOE NMR spectra were generated in 20 mM [U-2H]Tris–HCl, pH 7.1, using the following reaction mixtures: 0.1 mM ecRecA, 1 mM d(TAGC), 10 mM ATPγS, 6.7 mM MgCl2, and 150 mM NaCl; 0.05 mM hsRad51, 0.5 mM d(TAGC), 10 mM AMPPPN, 5 mM MgCl2, and 100 mM NaCl; 0.1 mM ecRecT, 1 mM d(TAGC), and 100 mM NaCl; 0.05 mM ttRecO, 0.5 mM d(TAGC), 5 mM MgCl2, and 150 mM NaCl; 0.05 mM Mhr1, 0.5 mM d(TAGC), 6.7 mM MgCl2, and 50 mM NaCl; 0.1 mM ttSSB and 1 mM d(TAGC); and 0.1 mM ecRecT, 1 mM r(UAGC), and 100 mM NaCl. All NMR measurements were obtained using a DRX600 or AVANCE600 spectrometer (Bruker BioSpin, Germany) with a cryoprobe at 298 K. The mixing time was 0.18 s. All two-dimensional data sets were processed and analyzed with AZARA version 2.7 and ANSIG version 3.3 for OpenGL (48). The assignment of TRNOE cross-peaks was performed as previously described (19).

Analysis of NMR Data and Structure Calculations—NOE-derived distance constraints were used for structure calculations of d(TAGC) bound to hsRad51, ecRecT, ttRecO, Mhr1, and ttSSB using the simulated annealing protocol of XPLOR-NIH (49, 50). NOE intensities were calibrated using those of C[H5]–C[H6] as a reference. The structural refinements were performed as previously described (19).

RESULTS

Structural Properties of ssDNA Bound to Mhr1 and Other Evolutionarily Distinct HP Proteins—To investigate whether the extended structure of ssDNA complexed with RecA is conserved for Mhr1 and other HP proteins, we analyzed the structure of a four-nucleotide ssDNA oligomer, d(TAGC), bound to each of five HP proteins (Mhr1, ecRecA, ecRecT, ttRecO, and hsRad51) using the TRNOE, an NMR method for analyzing the structure of small ligands bound to large molecules (51). The patterns for each of the TRNOE cross-peaks were very similar to that observed for the spectrum of ssDNA in the presence of RecA (19) (Fig. 1). Inter-residue cross-peaks were detected between H3′ of the sugar moiety and H8 of purine, or H6 of pyrimidine (H8/H6), whereas only weak, or no, inter-residue cross-peaks were detected between H1′ and H8/H6. We also observed relatively weak sequential H2′–H8/H6 and H2′–H8/H6 NOEs of comparable intensity (Fig. 1, B–F). In the case of ssDNA bound to ATP-independent HP proteins in the absence of nucleotide cofactors, the TRNOE spectra of ecRecT, ttRecO, and Mhr1 (Fig. 1, D–F) were very similar to those of ecRecA and hsRad51 in the presence of ATP analogues (Fig. 1, B and C, respectively). The pattern of cross-peaks indicated that ssDNA adopts an extended structure. As a negative control, the TRNOE spectrum of ttSSB, which can bind ssDNA but is not a HP protein, was distinct from those of the HP proteins. In particular, significant base-base inter-residue cross-peaks were observed between H6 and H8 in the ttSSB-ssDNA spectrum (Fig. 2A), whereas very weak, or no, base-base inter-residue cross-peaks were observed in the HP protein-ssDNA spectra (Fig. 2, B–F). These results indicated that the ssDNA bound to each of the HP proteins has structural properties similar to that of ssDNA bound to RecA and that base stacking was abolished. In agreement with these structural differences, ttSSB was less effective in a base unstacking assay than the HP proteins, whereas ecRecA, ecRecT, ttRecO, and Mhr1 mediated unstacking of ssDNA bases (supplemental Fig. S3). These findings indicate that base unstacking is a common feature of HP protein binding.

The Three-dimensional Structure of ssDNA Bound to HP Proteins—To determine the precise tertiary structures of ssDNA bound to each of the HP proteins, we carried out structure calculations based on distance restraints derived from the TRNOE spectra. We performed structure calculations using the simulated annealing protocol of XPLOR-NIH (49, 50). The structures were well defined (Fig. 3A, NMR and refinement statistics for these structure determinations are described in supplemental Table S1). Superimposition of the 10 lowest...
energy structures of Mhr1-, ecRecT-, ttRecO-, and hsRad51-bound ssDNA are shown in Fig. 3A. HsRad51-bound ssDNA had a poorer fit for the 5' region of d(TACG) than for the 3' region, whereas the 5' region exhibited a better fit when complexed with RecA (19). This may be due to differences in the polarity of the strand exchange reaction between hsRad51 (3' to 5'; see Ref. 52) and ecRecA (5' to 3'; see Ref. 10). Fig. 3B illustrates the refined molecular models of the neighboring two residues of d(TACG) bound to each of the HP proteins. These structures were very similar to each other. Interestingly, normal stacking of adjacent bases through van der Waals contacts, as seen in B-form DNA, was absent in all structures (Fig. 3B), and the 2'-methylene moiety of deoxyribose was positioned above the base of the following residue (Fig. 3C, i).

Characteristic Features of the Extended ssDNA Structure—The unique extended and unstacked structure of ssDNA would facilitate base rotation during HP. In contrast to ssDNA bound to HP proteins, there is no 2'-methylene-base interaction in B-form DNA. Although the methylene moiety of Mhr1- or ecRecT-bound ssDNA was positioned above the edge of the base, hydrophobic deoxyribose-base interactions were still maintained. In the crystal structure of the RecA-ssDNA complex, the second base of the “triplet” exhibited a similar configuration of methylene in relation to the following base (Fig. 3C,...
As a result, the interconversion of sugar puckering (3' endo versus 2' endo) is expected to induce an almost horizontal rotation of bases, because the plane of the base is almost orthogonal to that of the sugar ring. The base rotation facilitates scanning to find a complementary sequence of the ssDNA with dsDNA engaged in an HP reaction. The finding of a complementary sequence would result in a base pair switch to accomplish HP.

Comparison of the Solution Structures with Crystal Structures of ssDNA Bound to RecA—Because the crystal structure of the RecA-ssDNA complex was recently resolved (21), we compared the solution structures of ssDNA bound to RecA obtained by NMR with the crystal structure. Superimposition of the solution structure of RecA-bound d(TACG) and the crystal structure of the triplet region in the RecA5(dT)15 complex showed that the two structures are similar (Fig. 4A). The rise parameter was calculated for each base step with the program 3DNA (53) used by Chen et al. (21) (Table 1). The rise parameters in the triplet region of the crystal structure ranged from 3.83 to 4.66 Å. We re-evaluated the rise parameters for the solution structure using the same program, although it was already reported to be nearly 5 Å (19), and found that they ranged from 3.86 to 5.35 Å. Because the rise parameter of the “inter-triplet” region ranged from 7.14 to 7.84 Å, the solution structure strongly reflects the triplet region. In addition, we estimated whether the NMR data could be explained as an ensemble of the crystal structure. Based on the crystal structure, we constructed three different d(TACG) structures that correspond to the three possible binding modes to the RecA filament (Fig. 4B). Of the NOE-derived distance constraints that were used for the d(TACG) structure calculation, 93% were satisfied by inter-proton distances derived from at least one of the three models within the violation criterion (0.5 Å). Assuming that the RecA-bound DNA was in conformational exchange among the three models, the NOE-derived distance constraints were best explained by using weighted average distances,

\[
(d = \left[ \sum_m w_m d_m^{-6} \right]^{-1/6})
\]

(Eq. 1)

when models 1, 2, and 3 exist at ratios of 16, 82, and 2%, respectively (Fig. 4B). This result also indicates that the NMR data strongly reflect the structure of the triplet region, because both models 1 and 2 contain the triplet region. Although the method used in the structure calculation assumed that d(TACG) had a single conformation that might have biased the rise parameter toward larger values, we think that the solution structure reproduced the structural features of the triplet region well. In the next section, to eliminate any bias caused by the conformational exchange, we examine structural features by focusing on certain characteristic NOE signals. It is also possible that the differences in the rise parameters were caused by differences in environmental factors, i.e., crystal and solution. Indeed, molecular dynamics simulations showed that the crystal structure of the RecA-ssDNA complex would fluctuate with root mean square deviation values of about 2 Å in an aqueous environment (see supplemental Fig. S4 for details).

Comparison of the Triplet Structure with B-form DNA—Although the triplet structure is similar to B-form DNA (Fig. 4C), detailed comparison of the inter-residue distances between the ribose and pyrimidine base moieties (5’ to 3’) revealed a clear difference between these structures (Table 2). The hallmarks of B-form DNA are a short \(d_1\) (H1'; H6) and a long \(d_2\) (H3'; H6). The D1 and D2 distances between adjacent bases in the triplet in the crystal structure and in the solution structure of RecA-bound ssDNA revealed a long \(d_1\) (H1'; H6) and a short \(d_2\) (H3'; H6). Because the distances between the bases in the inter-trip-
let region (D3) were longer than 7 Å, the contribution from a conformation like that of model 3 to these NOEs can be ignored. These observations indicated that similar structural features are present in the crystal structure of RecA5-(dT)15 and in the solution structure of RecA-d(TACG), while these features are clearly different from those of B-form DNA. Although normal base stacking is apparent in B-form DNA, base stacking is abolished in RecA-bound ssDNA in the crystal and solution structures (Fig. 4C). In addition, all solution structures of ssDNA bound to HP proteins showed greater similarity to the triplet region than to the inter-triplet region (details in Table 2). These results strongly suggest that a unique extended ssDNA structure, as shown in the triplet region, is important for HP and that HP is catalyzed by this universally adopted structure, independently of the species of HP proteins that participate in the reaction.

RNA Is Unable to Adopt an Extended Structure—In contrast to ssDNA, an extended RNA structure with a similar configuration of ribose relative to the following base was impossible because RNA contains a large hydrophilic hydroxyl group at the 2’ position of the sugar ring (Fig. 5). We performed TRNOE analysis of ecRecT in the presence of a four-nucleotide RNA oligomer, r(UACG). Unlike RecA, which does not bind to RNA, ecRecT bound to r(UACG). We found that the spectrum contained cross-peaks that corresponded to base stacking (Fig. 2G), similar to that observed for the ttSSB-ssDNA structure (Fig. 2A). These results demonstrated that despite being able to bind to a HP protein, RNA is unable to adopt an extended structure. Thus, the extended structure of ssDNA bound to an HP protein depends strongly on the chemical characteristics of DNA.

DISCUSSION

It is known that RecA and Rad51 proteins untwist dsDNA during ATP-dependent HP reactions (see Introduction), but in previous work (30) we found that Mhr1 promotes HP without the net untwisting of dsDNA. In addition to Mhr1, some ATP-independent HP proteins with various quaternary structures different from those of RecA, Rad51, or Mhr1 were found in
The most unique feature of the common DNA structure is a hydrophobic interaction between the 2′ methylene moiety of deoxyribose and the following base resulting from base-base stacking (Fig. 3C). Some primitive organisms retain RNA genomes, in which HR rarely occurs (54), if at all, and recombination is mediated by a copy-choice mechanism (replicative template switch) that does not include HP (55, 56). RNA is unlikely to adopt an extended structure because of the presence of a bulky hydrophilic hydroxyl group at the 2′ position of the ribose moiety (Fig. 5). Experimentally, RNA poorly binds to RecA (19), and even RNA that bound to an HP protein, ecRecT, did not adopt an extended structure (Fig. 2G). This explains why recombination of the RNA genome is performed by a copy-choice mechanism. Because this type of HR requires single-stranded parental RNAs, it represents a significant challenge to genomic stability. On the other hand, the structural properties of DNA are useful for not only maintaining the integrity of the genome, but also for promoting HP, which is a well-regulated yet highly dynamic process and allows for adaptation to changing environments. Thus, DNA has a critical advantage over RNA as a carrier of genetic information (Fig. 6).

The extended DNA structure containing a hydrophobic interaction between the 2′ methylene moiety of deoxyribose and the following base was originally found in ssDNA bound to ecRecA (19). Our current finding indicates that the extended DNA structure is not specific to RecA, but suggests that it is fundamentally important in HR; i.e., unrelated HP proteins employ a common mechanism to catalyze HP at the DNA structural level, independent of their quaternary structures (Fig. 7). This suggestion is somewhat surprising, because HP proteins require a topological state of the dsDNA substrate unique to each HP protein, and because required topological states have been considered to reflect the specificity of each DNA reaction.

The common mechanism may be a base rotation coupled with the interconversion of sugar pucker, which we postulated in our previous work (20). In the extended ssDNA conformation, the distance between adjacent bases is longer than that in normal B-form DNA, which disrupts base stacking (Fig. 4C) and permits bases to rotate. These extended DNA structures are also found in the crystal structure of the RecA-ssDNA complex (see Tables 1 and 2 and Fig. 3). Superimposition of the solution structures of RecA-bound d(TAGC) revealed by NMR and the triplet region of the crystal structure of ssDNA in the RecA5-(dT)15 complex showed that the two structures are similar (Fig. 4A). These structures have clearly different features from B-form DNA, but enable each base to engage in Watson-Crick base pairing. Although the inter-triplet region would also be important for HR...
mediated by RecA, it is likely to be specific for RecA because it is stabilized by the interaction with the L2 loop (21). The extended structure that we revealed by NMR was observed for all HP proteins tested, probably because its stability is an intrinsic structural property of DNA itself.

Because HP involves base pair switches that require extended spaces between base pairs, an extended conformation is also necessary for dsDNA. The extension of dsDNA unavoidably induces the untwisting of the double helix, which generates positive supercoils in the neighboring DNA regions. Consistent with this requirement, negative supercoils of substrate dsDNA, which cancel the positive supercoiling, extensively stimulate HP by RecA and Rad51, as described under the Introduction. The binding of RecT also introduces positive supercoils into dsDNA (42). Thus, RecA, Rad51, and RecT are optimized to work well on negatively supercoiled dsDNA. Mhr1 promotes HP with relaxed closed circular dsDNA at a much higher efficiency than with negatively supercoiled dsDNA (30). Relaxed closed circular dsDNA accumulates topological constraints by either untwisting or twisting, indicating that Mhr1 catalyzes HP without a net topological change of the dsDNA substrate (30).

In addition, the product of Mhr1-catalyzed HP is not a D-loop that contains unpaired strands of parental dsDNA (thus, untwisted), but a stable homologous complex of dsDNA and ssDNA that does not involve untwisting of the parental dsDNA by even a single turn (30). A likely product is parallel triplex that contains recombinant base pairs between incoming ssDNA and the complementary strand from dsDNA, with the displaced strand lying in the major groove (57). This property is favorable to mtDNA, which is likely to be relaxed because of complex formation between mtDNA and a large amount of TFAM (or Abf2), as described under the Introduction. Thus, Mhr1 is opti-
mized for HP in mitochondria. Likewise, each HP protein is probably optimized to perform HP on dsDNA under local topological conditions. This may explain why multiple HP proteins with significantly different tertiary and quaternary structures arose during evolution (Fig. 7).

The topological states of the favorable dsDNA substrate for each HP protein are variable, as described above. Thus, each HP protein may use a different strategy to extend dsDNA to promote HP. RecA/Rad51 proteins form nucleoprotein filaments along DNA that extend dsDNA as well as ssDNA, associated with the untwisting of double helices and positive supercoiling of neighboring DNA regions, which is compensated for by negative supercoiling of the substrate dsDNA. RecT may use a similar strategy (see Ref. 42). The binding of dsDNA to Mhr1 does not generate net topological constraints, and we proposed that the extension of dsDNA-induced untwisting of a double helix is canceled by the simultaneous right-handed wrapping of dsDNA around Mhr1 (30). The initiation of right-handed wrapping is prevented by negative supercoiling of dsDNA, and, thus, negatively supercoiled dsDNA is an unfavorable substrate for Mhr1. The binding of closed circular dsDNA to hsRad52 induces negative supercoils (41). Because dsDNA-binding sites are located around a multimeric ring formed by Rad52, it was proposed that dsDNA wraps around the Rad52 ring in a right-handed configuration while simultaneously extending (and thus untwisting) the dsDNA (41), but, unlike Mhr1, the extent of negative supercoiling induced by the wrapping is greater than that sufficient to cancel the untwisting. Thus, each HP protein induces the extension of dsDNA during the HP reaction by a unique strategy.

Acknowledgments—We thank Drs. Hitoshi Kurumizaka and Feng Ling for providing the expression plasmids for hsRad51 and Mhr1, respectively.

REFERENCES

1. Shibata, T., DasGupta, C., Cunningham, R. P., and Radding, C. M. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 1638–1642
2. McEntee, K., Weinstock, G. M., and Lehman, I. R. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 2615–2619
3. Cox, M. M., and Lehman, I. R. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 3433–3437
4. Kahn, R., Cunningham, R. P., DasGupta, C., and Radding, C. M. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 4786–4790
5. Kuzminov, A. (1999) Microbiol. Mol. Biol. Rev. 63, 751–813
6. Kowalczykowski, S. C. (2000) Trends Biochem. Sci. 25, 156–165
7. Shibata, T., Nishinaka, T., Mikawa, T., Aihara, H., Kurumizaka, H., Yokoyama, S., and Ito, Y. (2001) Proc. Natl. Acad. Sci. U.S.A. 98, 8425–8432
8. West, S. C. (2003) Nat. Rev. Mol. Cell Biol. 4, 435–445
9. Krogg, B. O., and Symington, L. S. (2004) Annu. Rev. Genet. 38, 233–271
10. Cox, M. M. (2007) Nat. Rev. Mol. Cell Biol. 8, 127–138
11. Shibata, T., Cunningham, R. P., DasGupta, C., and Radding, C. M. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 5100–5104
12. Egel, R. (2007) DNA Repair 6, 669–675
13. Flory, J., Tsang, S. S., and Muniyappa, K. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 7026–7030
14. Stasiak, A., Di Capua, E., and Koller, T. (1981) J. Mol. Biol. 151, 557–564
15. Dunn, K., Chrysopegos, S., and Griffith, J. (1982) Cell 28, 757–765
16. Heuser, J., and Griffith, J. (1989) J. Mol. Biol. 210, 473–484
17. Yu, X., and Egelman, E. H. (1992) J. Mol. Biol. 227, 334–346
18. Stasiak, A., and Di Capua, E. (1982) Nature 299, 185–186
19. Nishinaka, T., Ito, Y., Yokoyama, S., and Shibata, T. (1997) Proc. Natl. Acad. Sci. U.S.A. 94, 6623–6628
20. Nishinaka, T., Shinohara, A., Ito, Y., Yokoyama, S., and Shibata, T. (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 11071–11076
21. Chen, Z., Yang, H., and Pavletich, N. P. (2008) Nature 453, 489–494
22. Cunningham, R. P., Shibata, T., DasGupta, C., and Radding, C. M. (1979) Nature 281, 191–195
23. Shibata, T., Cunningham, R. P., and Radding, C. M. (1981) J. Biol. Chem. 256, 7557–7564
24. Shibata, T., DasGupta, C., Cunningham, R. P., Williams, J. G., Osber, L., and Radding, C. M. (1981) J. Biol. Chem. 256, 7565–7572
25. Van Komen, S., Petukhova, G., Sigurdsson, S., Stratton, S., and Sung, P. (2000) Mol. Cell 6, 563–572
26. Rouviere-Yaniv, J., Yaniv, M., and Germond, J. E. (1979) Cell 17, 265–274
27. Caron, F., Jacqu, C., and Rouviere-Yaniv, J. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 4265–4269
28. Fisher, R. P., Lisowsky, T., Parisi, M. A., and Clayton, D. A. (1992) J. Biol. Chem. 267, 3358–3367
29. Ling, F., and Shibata, T. (2002) EMBO J. 21, 4730–4740
30. Ling, F., Yoshida, M., and Shibata, T. (2009) J. Biol. Chem. 284, 9341–9353
31. Kurumizaka, H., Ikawa, S., Nakada, M., Eda, K., Kagawa, W., Takada, M., Takeda, S., Yokoyama, S., and Shibata, T. (2001) Proc. Natl. Acad. Sci. U.S.A. 98, 5538–5543
32. Kagawa, W., Kurumizaka, H., Ikawa, S., Yokoyama, S., and Shibata, T. (2001) J. Biol. Chem. 276, 35201–35208
33. Rybalchenko, N., Golub, E. I., Bi, B., and Radding, C. M. (2004) Proc. Natl. Acad. Sci. U.S.A. 101, 17056–17060
34. Noiro, P., and Kolodner, R. D. (1998) J. Biol. Chem. 273, 12274–12280
35. Luisi-DeLuca, C. (1995) J. Bacteriol. 177, 566–572
36. Mazloum, N., Zhou, Q., and Holloman, W. K. (2008) Proc. Natl. Acad. Sci. U.S.A. 105, 524–529
37. Inoue, J., Honda, M., Ikawa, S., Shibata, T., and Mikawa, T. (2008) Nucleic Acids Res. 36, 94–109
38. Leiros, I., Timmins, J., Hall, D. R., and McSweeney, S. (2005) EMBO J. 24, 906–918
39. Passy, S. I., Yu, X., Li, Z., Radding, C. M., and Egelman, E. H. (1999) Proc. Natl. Acad. Sci. U.S.A. 96, 4279–4284
40. Thresher, R. I., Makrov, A. M., Hall, S. D., Kolodner, R., and Griffith, J. D. (1995) J. Mol. Biol. 254, 364–371
41. Kagawa, W., Kagawa, A., Saito, K., Ikawa, S., Shibata, T., Kurumizaka, H.,...
and Yokoyama, S. (2008) *J. Biol. Chem.* **283**, 24264–24273
42. Noirot, P., Gupta, R. C., Radding, C. M., and Kolodner, R. D. (2003) *EMBO J.* **22**, 324–334
43. Clark, A. J., Sharma, V., Brenowitz, S., Chu, C. C., Sandler, S., Satin, L., Templin, A., Berger, I., and Cohen, A. (1993) *J. Bacteriol.* **175**, 7673–7682
44. Mikawa, T., Masui, R., Ogawa, T., Ogawa, H., and Kuramitsu, S. (1995) *J. Mol. Biol.* **250**, 471–483
45. Inoue, J., Shigemori, Y., and Mikawa, T. (2006) *Nucleic Acids Res.* **34**, e69
46. Matsuo, Y., Sakane, I., Takizawa, Y., Takahashi, M., and Kurumizaka, H. (2006) *FEBS J.* **273**, 3148–3159
47. Yoshimasu, M., Aihara, H., Ito, Y., Rajesh, S., Ishibe, S., Mikawa, T., Yokoyama, S., and Shibata, T. (2003) *Nucleic Acids Res.* **31**, 1735–1743
48. Kraulis, P. J. (1989) *J. Magn. Reson.* **84**, 627–633
49. Schwieters, C. D., Kuszewski, J. J., Tjandra, N., and Clore, G. M. (2003) *J. Magn. Reson.* **160**, 65–73
50. Schwieters, C. D., Kuszewski, J. J., and Clore, G. M. (2006) *Prog. NMR Spectrosc.* **48**, 47–62
51. Post, C. B. (2003) *Curr. Opin. Struct. Biol.* **13**, 581–588
52. Baumann, P., and West, S. C. (1997) *EMBO J.* **16**, 5198–5206
53. Lu, X. J., and Olson, W. K. (2003) *Nucleic Acids Res.* **31**, 5108–5121
54. King, A. M., McCahon, D., Slade, W. R., and Newman, J. W. (1982) *Cell* **29**, 921–928
55. Lai, M. M. (1992) *Microbiol. Rev.* **56**, 61–79
56. Chetverin, A. B. (1999) *FEBS Lett.* **460**, 1–5
57. Vlieghe, D., Van Meervelt, L., Dautant, A., Gallois, B., Précigoux, G., and Kennard, O. (1996) *Science* **273**, 1702–1705
58. Kagawa, W., Kurumizaka, H., Ishitani, R., Fukai, S., Nureki, O., Shibata, T., and Yokoyama, S. (2002) *Mol. Cell* **10**, 359–371
59. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., and Ferrin, T. E. (2004) *J. Comput. Chem.* **25**, 1605–1612