Mechanisms of Maintaining Genetic Stability by Homologous Recombination

Yoshizumi Ishino,*[,†,‡] Tatsuya Nishino,[§,||] and Kosuke Morikawa*[,§]

Department of Genetic Resources Technology, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Fukukoka-shi, Fukuoka 812-8581, Japan, and Department of Molecular Biology and Department of Structural Biology, Biomolecular Engineering Research Institute (BERI), 6-2-3 Furuedai, Suita, Osaka 565-0874, Japan

Received June 20, 2005

Contents

1. Introduction

It is essential for living organisms to replicate and transfer the genetic information in the genome precisely from parents to offspring. For this purpose, many repair systems have been established in cells during evolution, and they function to ensure genomic integrity, as described in this special issue. The proofreading activity of DNA polymerases directly functions in accurate DNA strand synthesis.[1] In addition, several repair systems operate at the damaged points and are controlled by the cell cycle checkpoint system.[2,3] The well-known DNA repair systems, such as nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), homologous recombination repair (HRR), and end joining, are conserved from prokaryotes to eukaryotes.[3]

In cells, accurate DNA replication is often blocked by a variety of intrinsic and extrinsic causes, including nucleotide lesions, backbone gaps, and other anomalous regions, as well as by topological stress, tightly bound proteins, aberrant DNA structures, and many DNA-damaging agents.[4−6] These blocks during DNA replication lead to serious diseases, including cancer. To overcome stalled fork progression, cells utilize replication-coupled repair systems. DNA polymerase-dependent translesion synthesis and bypass or collapse/restart of the stalled fork are two major pathways.[4−13] In the latter case, homologous recombination (HR) plays a major role in the process, where a four-way junction is formed by unwinding the stalled fork, so that fork regression can allow the newly synthesized strands and the parental strands to anneal. This four-way junction structure is just like a Holliday junction (HJ) intermediate in HR, as originally identified by Holliday.[14] This intermediate could be processed by homologous recombination either after or without cleavage. Current models of the HR for double-strand break repair and replication fork repair are shown in parts A and B, respectively, of Figure 1. The fork structure could be reassembled by reprogression after DNA strand systhesis at the lesion site using the other nascent DNA strand as the template or by cleavage at the junction point with HJ resolvases. The HJ-like four-way junction structures formed from stalled replication forks in the cells have been conditionally observed by electron microscopy.[15,16] Alternatively, the stalled fork can be directly cleaved by structure-specific endonucleases. When the DNA strands of the four-way junction or fork structure are cleaved by these endonucleases, the fork can be reconstructed by homologous recombination starting from the free DNA ends (Figure 1B). Thus, it is very important to understand how the physical and functional interactions between the replication and recombination enzymes maintain faithful chromosomal duplication.

The coupling mechanisms between replication and recombination have been extensively analyzed in *Escherichia coli*.[10,17,18] The four-way junction, produced by replication fork regression and referred to as a "chicken foot", can be processed by cleavage in two opposing strands at the junction point by the HJ resolvase, RuvC. Alternatively, the HJ can be processed without collapse of the fork by cleavage. The free end of the HJ produced at the stalled fork can be digested by the RecBCD exonuclease to reform the fork structure,

* To whom correspondence should be addressed. Yoshizumi Ishino, Prof., Kyushu University: phone, +81-92-642-4217; fax, +81-92-642-3051; e-mail, ishino@agr.kyushu-u.ac.jp. Kosuke Morikawa, Dr.: phone, +81-6-6872-8211; fax, +81-6-6872-8210; e-mail, morikako@protein.osaka-u.ac.jp.
† Kyushu University.
‡ Department of Molecular Biology, Biomolecular Engineering Research Institute (BERI).
§ Department of Structural Biology, Biomolecular Engineering Research Institute (BERI).
|| Present address: Research Institute of Molecular Pathology, Dr. Bohrgasse 7, A-1030 Vienna, Austria.

10.1021/cr0404803 CCC: $59.00
Published on Web 12/30/2005

Yoshizumi Ishino graduated from the faculty of Pharmaceutical Science, Osaka University, Japan, and earned B.S. (1981) and M.S. (1983) degrees with research on structure and functions of restriction endonucleases. He studied DNA ligase and the *lig* gene from *E. coli* at Research Institute for Microbial Diseases, Osaka University, where he earned his Ph.D (1986). He worked in Prof. Dieter Soll's lab at Yale University as a postdoctoral fellow for two years (1987–1989) doing research on translation-related enzymes, and then came back to Bioproducts Development center in Takara Shuzo, Japan. He became a senior research scientist in Biotechnology Research Laboratories in Takara Shuzo. He moved to Biomolecular Engineering Research Institute (BERI), a national project funded industry ministry and 18 companies, in 1996 and managed a research group on nucleic acids-related enzymes. In 2002, he was appointed a full professor of protein chemistry and engineering in Department of Genetic Resources Technology, Kyushu University. His main research interests have been molecular mechanisms of DNA replication and recombinational repair in Archaea, the third domain of life.

Tatsuya Nishino studied biology at International Christian University in Tokyo. In 1995, he moved to Osaka University to start his diploma under Prof. Hideo Shinagawa to study molecular analysis of bacterial homologous recombination proteins. At the same time, he studied protein X-ray crystallography under Prof. Kosuke Morikawa, and he continued this work for his Ph.D. research. In 2000, he received a Ph.D. from Osaka University on the structural analyses of recombinational proteins. Since 2003, he has been working with Prof. Kim Nasmyth at Institute of Molecular Pathology in Vienna as a postdoctoral fellow. His current interest is to understand the molecular mechanism of cohesins involved in sister chromatid segregation.

Kosuke Morikawa was born in Tokyo, Japan, in 1942. He earned his B.S. (Pharmaceutical Sciences) and M.Sc. (Pharmaceutical Sciences) degrees at Tokyo University in 1967 and 1969, respectively. In 1972, he obtained his Ph.D. in Pharmaceutical Sciences at Tokyo University. After he had worked on studying nucleic acid structures by X-ray crystallography and spectroscopy as an assistant professor at Prof. M. Tsuboi's laboratory, Faculty of Pharmaceutical Sciences, Tokyo University, for three years, he moved to Prof. B. F. C. Clark's laboratory, Aarhus University, Denmark, and stayed there until at the end of 1977. There, he made a breakthrough for determining the crystal structure of *E. coli* elongation factor Tu. Then, he moved to MRC laboratory of Molecular Biology, Cambridge, England, to work in the group supervised jointly by Drs. H. Huxley and A. Klug. In 1980, he became an assistant professor, Faculty of Science, Kyoto University, Japan, and he stayed there until 1986. He was then appointed to be the director of the department of Structural Biology, PERI/BERI. Since 2004, he has been the director of BERI. His main research interests have been three-dimensional structural views of DNA–protein and ligand–receptor interactions.

which allows rebuilding of the replisome.[19] The RecG helicase specifically unwinds forked DNA structures to form the four-way junction *in vitro*, and it is believed to have a critical role in the interconversion of the fork and the four-way junction.[20] Formation of the four-way junction is not necessarily required if only the leading or the lagging strand is unwound to facilitate the repair and the resumption of DNA replication. The RecQ helicase can unwind various branched DNA structures *in vitro* as well,[21,22] and an *in vivo* study also showed that RecQ unwinds the lagging strand at stalled forks to promote the specific degradation of the strand by the RecJ exonuclease.[23] The processed fork can be stabilized by binding to RecA, with the help of the RecF, RecO, and RecR proteins.[24]

In eukaryotic cells, homologues of RuvABC and RecG have not been identified yet. However, the presence of the RecA homologue, Rad51, raises the possibility that Rad51-mediated homologous recombination could repair the stalled replication fork via a Holliday junction, in the same manner as proposed for *E. coli*. Homologues of RecQ helicase have also been identified in eukaryotes. In addition to the yeast Sgs1 (*Saccharomyces cerevisiae*) and Rqh1 (*Schizosaccharomyces pombe*) proteins, three human RecQ homologues, BML, WRN, and RECQ4, have been characterized. It is well-known that the genes encoding these three RecQ homologues are responsible for Blooms's, Werner's, and Rothmund−Thomson syndromes, respectively, which are hereditary diseases.[25,26]

It is not clear whether a pathway for restarting a stalled fork by cleavage exists in eukaryotes. The Mus81 protein, which is conserved in all eukaryotic organisms and shares sequence similarity with the XPF (mammals) and Rad1 (yeast) endonucleases, is a structure-specific endonuclease that works as a heterodimer with Eme1 (human and *S. pombe*) or Mms4 (*S. cerevisiae*).[27] This protein complex has recently been in the spotlight in the research field of DNA repair and recombination, where it has caused lots of debate regarding its *in vivo* function.[27,28] In addition to the issue of whether the conventional HJ resolvase exists in eukaryotic cells, the functions of the Mus81 nuclease complex are now one of the major topics in this field.

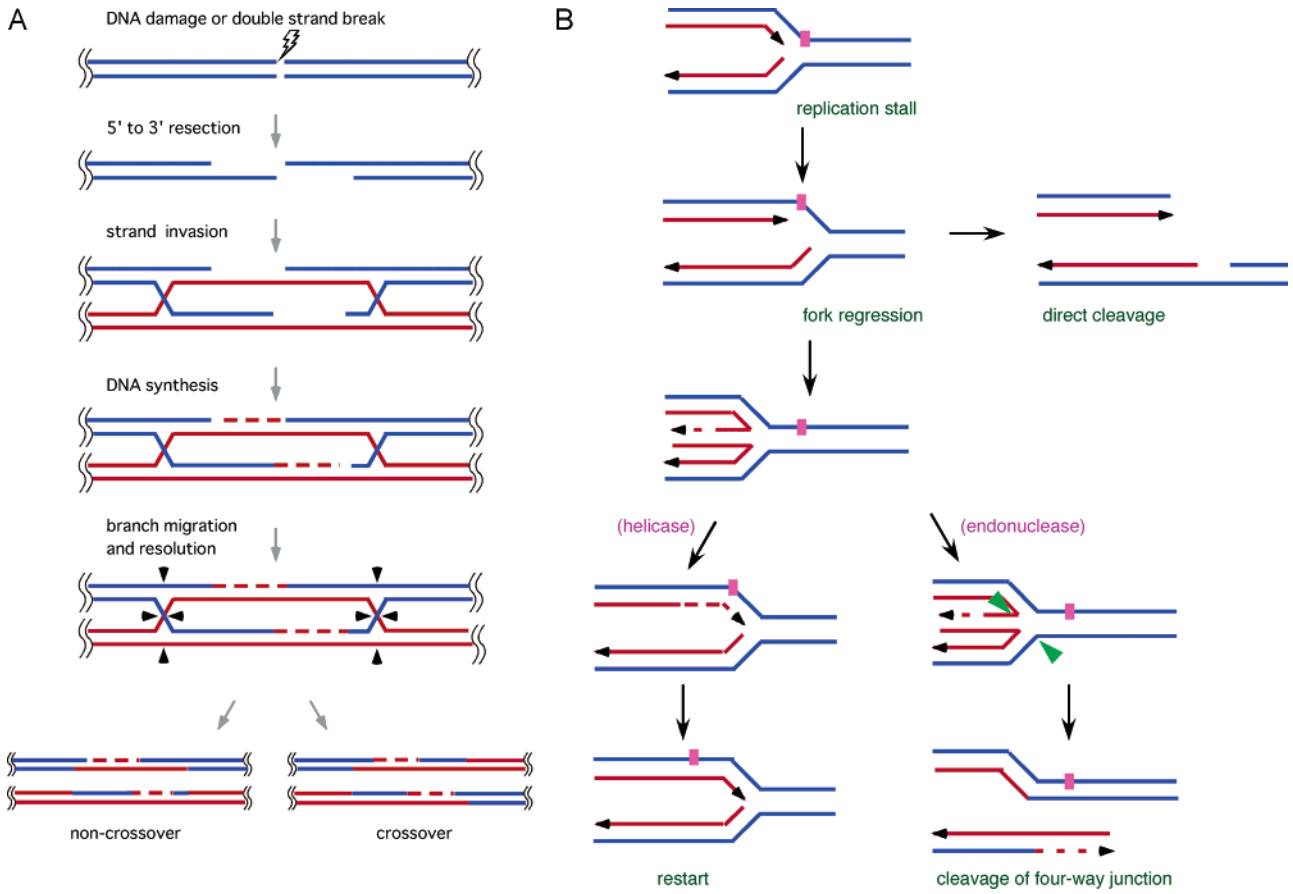

Figure 1. Homologous recombination and replication fork repair. (A) The current model of the repair process of the double-strand break by homologous recombination is shown. Holliday junction (HJ), the recombination intermediate, is made by a RecA-mediated strand exchange reaction. HJ is processed through migration and cleavage of the junction site. (B) Potential pathways of the stalled replication fork repair. Stalled replication forks can be restored with or without cleavage. A cleaved fork can be reestablished after homologous recombination. Proteins involved in these processes are discussed in this review article.

Archaea, the third domain of life,[29] is distinct from both Bacteria and Eukarya (Eukaryote). Whereas Archaea belong to the prokaryotes in terms of their cellular ultrastructure, they share strong similarities with Eukarya in their information processing pathways, including DNA replication, transcription, and translation,[30] and the archaeal processes provide useful model systems to understand the more complex mechanisms of their eukaryal equivalents. In addition, the hyperthermophilic Archaea produce very stable proteins, which are especially useful for three-dimensional (3D) structural analyses.

Several proteins involved in homologous recombination are also conserved between Eukarya and Archaea. For example, RadA and RadB, which play some important roles in homologous recombination,[31−33] have sequences more similar to that of eukaryotic Rad51 than to that of bacterial RecA.[34−35] Furthermore, *Pyrococcus furiosus* RPA (replication protein A), which is composed of three subunits, like the eukaryotic RPA, but differs from the bacterial SSB (single-stranded DNA binding protein), clearly stimulated a RadA-mediated strand exchange reaction.[36] With regard to the HJ processing, we identified an archaeal HJ resolvase and named it Hjc.[37] Hjc is an Archaea-specific protein, which lacks sequence and three-dimensional structural similarity to any other known HJ resolvase.[38,39]

To understand the molecular mechanism of the HJ processing in Archaea, we searched for proteins related to Hjc and found a novel endonuclease activity in *P. furiosus*. Identification of the gene corresponding to this activity revealed that the encoded protein consists of two distinct domains, which are similar to the DEAH helicase family and the XPF nuclease superfamily, respectively.[40] Biochemical characterization of each purified domain showed that these proteins have a specific affinity for branched DNA structures, including the replication fork. In fact, the N-terminal domain possesses an ATPase activity that was dramatically stimulated by fork-structured DNAs. The C-terminal domain has an endonuclease activity that specifically cleaved nicked, flapped, and fork-structured DNAs. Therefore, we designated this protein as Hef (helicase-associated endonuclease for fork-structured DNA).[40]

Hef is well-conserved in Euryarchaeota, a subdomain of Archaea, and may be a prototypical enzyme for the eukaryotic XPF/Rad1/Mus81 nuclease family, which functions in important repair processes for the stalled replication forks. Biochemical characterization showed that the helicase and endonuclease activities of Hef seem to work together for very efficient fork processing.[41] Interestingly, no homologous protein of Hef is found Crenarchaeota, the other subdomain of Archaea. Instead, the crenarchaeal organisms have a protein comprised of only the endonuclease domain, and its nuclease activity for the branched DNA is completely PCNA (proliferating cell nuclear antigen)-dependent.[42] Furthermore, the very important finding was published by two research group simultaneously this summer that the human and mouse orthologue of the archaeal Hef is involved in the repair pathway related to Fanconi anemia, a genetic disease characterized by genomic instability and cancer predisposi-

Table 1. Recombination Proteins in the Three Biological Domains

function	Bacteria	Eukarya	Archaea
DNA end processing	RecBCD	Rad50 Mre11 Nbs1, Xrs2	Rad50 Mre11
mediator	RecFOR	Rad52 Rad55/57	?
strand exchange	RecA	Rad51	RadA
ssDNA binding	SSB	RPA	RPA
branch migration	RuvAB	?	Hjm (?)
HJ resolution	RuvC	?	Hjc
specific helicase	RecQ	RecQ4, BLM WRN, Sgs1 Rqh1	Hjm
5′−3′ exonuclease	RecJ	?	?
junction endonuclease (helicase)	?	Mus81	Hef
replication restart	PriA	?	?

tion.[43,44] The elucidation of the concrete functions of the Hef protein in the replication fork repair is now a very hot issue.

The rapid accumulation of the structural data[45,46] and the biochemical characterizations of the protein factors are remarkable in the field of homologous recombination and recombinational repair. This review article focuses on the structures and functions of the proteins involved in the repair process for the restoration of stalled replication forks in the three biological domains (Table 1). The structural data at atomic resolution, combined with biochemical characterizations, have allowed us to create models for the molecular mechanism of each step in the recombinational repair systems.

2. Proteins Involved in the Homologous Recombination Process

2.1. Strand Exchange Reaction by RecA Family Proteins

The RecA protein is recognized as a recombinase that plays a crucial role in HJ formation by an ATP-dependent strand exchange reaction.[47] The recombinase initiates the formation of a nucleoprotein filament to mediate the interaction of the DNA strands with homologous sequences in an ATP-dependent manner. RecA family proteins, including bacterial RecA, eukaryotic Rad51, and archaeal RadA, have conserved structures and functions (Figure 2), and hence homologous recombination is believed to be a ubiquitous cellular mechanism. Structural characterizations have been published for many RecA family proteins from the three biological domains. RecA was observed to form right-handed helical filaments with ~6 molecules per turn.[48−54] Closed ring forms of RecA with 3- or 6-fold symmetry have also been reported.[55] Eukaryotic Rad51 filament structures were observed as extended or compressed forms in the presence of ATP or ATPγS.[56,57] Filaments and ring forms of archaeal RadAs were also observed by electron microscopy (EM).[32,58,59] The crystal structures of the filament[60] and the heptameric ring[61] of the archaeal RadA have also been reported. These structural studies suggested that the strand exchange activity of the RecA family proteins is closely related to their conformational flexibility, which is controlled by ATP binding and hydrolysis. At least four different functional states of RecA−DNA nucleoprotein filaments exist with different solution conditions, and they are designated O, Ac, Ao, and P.[47,62] Although many structural reports have been published, as described above, scant information exists regarding the detailed mechanism of homologous strand exchange. Recently, a strand exchange scheme has been reported on the basis of the high-resolution extended helical structure of the *Methanococcus voltae* RadA crystal.[63,64] Thus, the atomic structures of the RecA family proteins in complex with DNA are required for a full understanding of this reaction mechanism.

2.2. RecBCD as a Multifunctional Protein Complex

The RecBCD pathway is very major as the main route in the processes of double-strand break (DSB) repair. This protein complex produces a 3′-terminated ssDNA molecule coated with RecA protein at double-strand break sites, by binding to the blunt end of dsDNA followed by unwinding and cleavage of one strand.[65] The activities of the RecBCD enzyme are regulated by Chi sites (5′-GCTGGTGG-3′), which are well-characterized recombination hot spots in *E. coli*. The RecBCD complex (330 kDa) has helicase, nuclease, and RecA-loading activities. These functions of RecBCD are attributed to the three subunits, which are RecB, a 3′−5′ helicase and nuclease,[66] RecC, which recognizes Chi sequences,[67] and RecD, a 5′−3′ helicase.[68] The RecBCD complex progresses along duplex DNA using the bipolar motors of RecB and RecD, and the 3′-tail is processively digested. The crystal structure of RecBCD bound to a blunt-ended duplex DNA has recently been determined.[69] In this crystal structure, the DNA duplex is split across RecC to create a forklike structure, in which each separated strand heads toward different helicase motors. There is a "pin" structure that protrudes from the surface of RecC, and it serves to split the duplex DNA. Both RecB and RecD have the structural fold conserved in Superfamily 1 (SF1) helicases. Notably, this RecD structure is the first example of a 5′−3′ helicase belonging to SF1. It is even more interesting that RecC adopts the same structure as that of the canonical SF1 helicases, despite the lack of both conserved sequence motifs and helicase activity. RecC may have evolved from a helicase ancestor and lost the unwinding activity during evolution. This structural study has revealed the mechanism of how the complex processes DNA ends and recognizes a recombinational hot spot.

RecBCD contributes to converting the HJ, formed by regressing the replication fork, to the normal functional fork structure simply by digesting the nascent duplex DNA from the terminus. It is predicted that this highly processive helicase/nuclease would easily cleave one arm of the Holliday junction produced by fork regression, to reestablish a fork structure.[19] RecBCD, in combination with RecA, could also process the DNA terminus made by the RuvC digestion, to initiate recombination with the intact sister duplex. The RecA-mediated strand exchange creates a D-loop, and replication restarts through primosome assembly, as described below.

2.3. RPA Stimulates the Strand Exchange Reaction by RecA Family Proteins

Single-stranded DNA is a common intermediate in the various processes of DNA metabolism. Bacterial ssDNA binding protein (SSB) and eukaryotic replication protein A (RPA) play essential roles in DNA replication, recombination, and repair by binding to protect the ssDNA regions

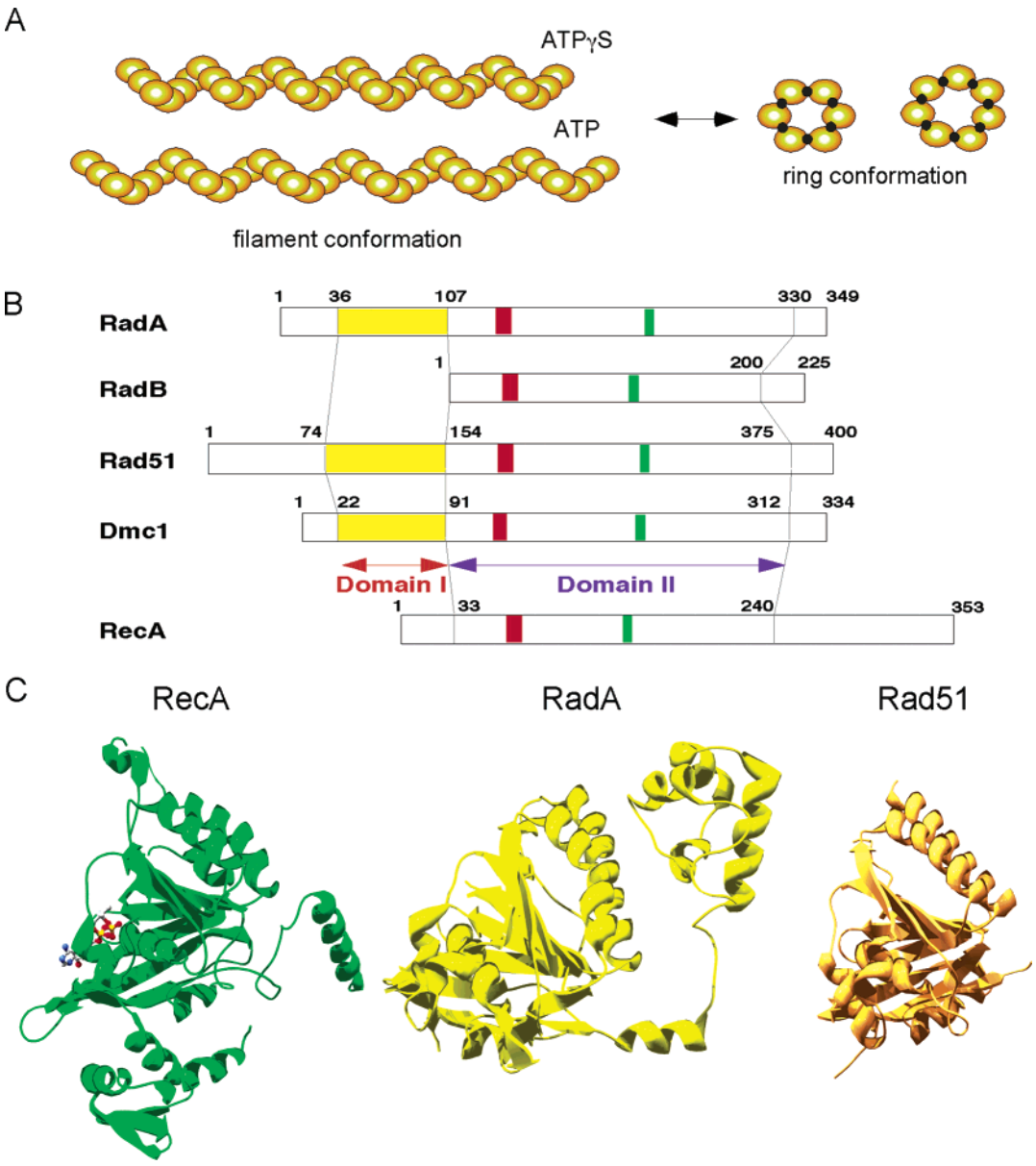

Figure 2. Structural view of RecA/RadA/Rad51 recombinase. (A) Two different structural states of the RecA proteins. The structural states are affected by the protein concentration, the bound nucleotide state (ATP, ADP, or no nucleotide), and the presence/absence of DNA. (B) Schematic drawings of RecA, RadA, and Rad51. Conserved domains among the three protein family members are designated as domain II. This region contains the ATP binding/hydrolysis site as well as the oligomerization domain. The red and green boxes indicate the Walker A and B motifs, respectively. Domain I (yellow box) is conserved only in Eukaryotes and Archaea. The amino acid residue numbers of each protein are derived from *P. furiosus* RadA and RadB, *S. cerevisiae* Rad51 and Dmc1, and *E. coli* RecA. (C) Crystal structures of the core domains of RecA (*Mycobacterium tuberculosis*; left), RadA (*P. furiosus*; middle), and Rad51 (human; right). Notice that all three proteins share a similar architecture. ADP/AlF_4, an ATP analogue, shown in a ball-and-stick model, is bound to the RecA nucleotide binding pocket.

from nucleases and DNA-damaging agents, and also by keeping them from forming inhibitory second structures.[70−72] SSB and RPA bind to ssDNA as a homotetramer and a heterotrimer, respectively (Figure 3). Despite the low sequence similarity between SSB and RPA, the three-dimensional structures revealed that they share a similar architecture, which contains an oligonucleotide/oligosaccharide binding (OB) fold.[73] In bacterial SSB, each 20 kDa subunit has one OB fold. On the other hand, RPA70, with three RPA subunits, contains two tandem repeats of the OB fold, while the other two subunits, RPA32 and RPA14, each possess one OB fold, respectively (Figure 3). RPA is also found in Archaea. For example, *P. furiosus* has a hetero-oligomeric RPA composed of RPA41, RPA32, and RPA14.[36] These ssDNA binding proteins bind to ssDNA, thereby stabilizing the unwound DNA and facilitating the assembly of the complex through direct interactions with various other protein factors. RPA stimulates the eukaryotic Rad51[74,75] and the archaeal RadA[36]-mediated strand-exchange reactions *in vitro*.

The crystal structures of the apo form[76] and the DNA-bound form[77] of human RPA revealed the mechanism of OB-fold binding to ssDNA (Figure 3). The two loops in the OB fold are flexible in the absence of DNA, and the binding pocket is open. Upon the loading of ssDNA, the loops clamp down on it and stabilize the complex in the closed conformation. The crystal structures of *E. coli* SSB in the presence and absence of ssDNA were reported, and their DNA binding

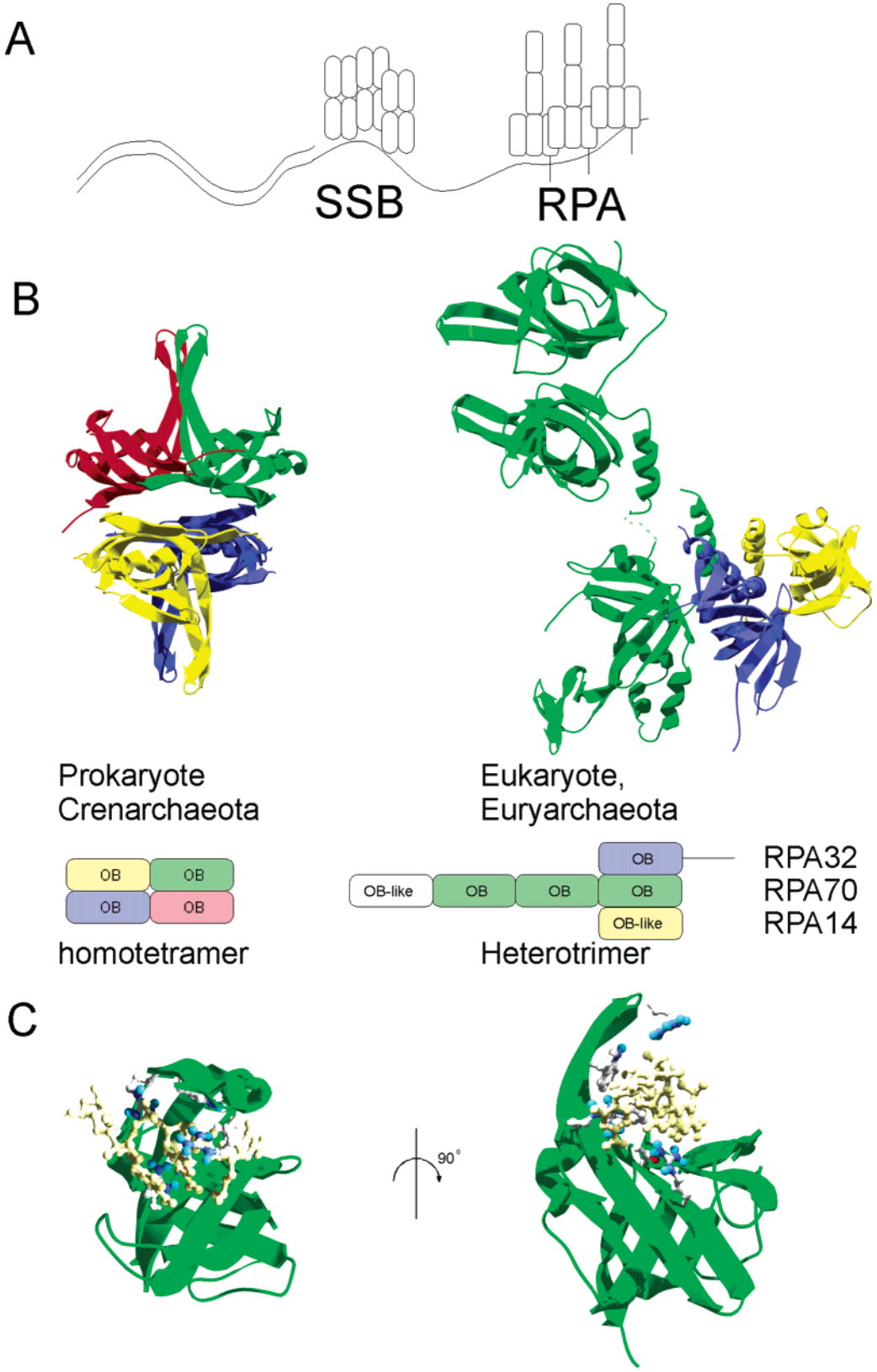

Figure 3. Structure and binding mode of the ssDNA binding proteins SSB and RPA. (A) Schematic drawing of SSB/RPA binding to a ssDNA region. SSB or RPA cooperatively binds and protects ssDNA regions from degradation by nucleases or helicases. (B) Crystal structures of apo SSB (*E. coli*; left) and RPA (*human*; right) oligomers. Each subunit in SSB and RPA is colored differently. In the RPA complex, which is constructed by combining two independent crystal structures through the flexible linker, RPA70, RPA32, and RPA14 are indicated by green, blue, and yellow. A schematic diagram of the subunit oligomerization is shown at the bottom. (C) Structure of the ssDNA−RPA70 OB fold. The protein is shown as a ribbon diagram, and the bound DNA is depicted by a ball-and-stick model (light yellow). Amino acid residues that recognize ssDNA are shown in ball-and-stick models.

modes were discussed.[78,79] Recently, the crystal structure of the SSB from *Deinococcus radiodurans*, a hyperradiotolerant bacterium, has been determined.[80] The *D. radiodurans* SSB contains two OB folds per monomer and assembles a four-

OB fold arrangement by means of symmetric dimerization.

Intriguingly, some organisms in the Euryarchaea, a subdomain of Archaea, including *P. furiosus*, have the eukaryote-like RPA, as described above, but the SSBs in the Crenarchaeota, the other subdomain of Archaea, are much more similar to the bacterial SSB proteins, with a single OB fold for DNA binding and a flexible C-terminal tail.[81] The crystal structure of the SSB protein from the Crenarchaeote, *Sulfolobus solfataricus*, unexpectedly showed a striking similarity to the DNA binding domains of human RPA, supporting the idea of the close relationship between Archaea and Eukarya.[82]

There are six OB folds, four in RPA70 and one each in RPA32 and RPA14, in total in the RPA complex. RPA binds ssDNA using four OB-folds, three in RPA70 and one in RPA32, which correspond to the four OB folds in the bacterial SSB. The crystal structures of human RPA32 and RAP14 have also been determined, and the OB folds within both subunits are structurally conserved.[83] Furthermore, the crystal structure of the RPA trimeric core, comprising the C-terminal DNA binding domain of RPA70, the central DNA binding domain of RPA32, and the entire RPA14 molecule, has been reported.[84] On the basis of these structural analyses, a sequential binding model of RPA with a hierarchical assembly from 5′ to 3′ of the ssDNA has been proposed. EM observations showed that RPA changes its conformation from a globular to an elongated form upon DNA binding.[85] The crystal structure of the RPA complex including ssDNA will help us to understand the precise mechanism of the specific ssDNA binding by RPA and the expression of its functions with various DNA structures.

2.4. RuvABC Proteins for HJ Processing

Resolution of the HJ intermediate in the homologous recombination process is mainly performed by the RuvABC proteins in Bacteria[86,87] (Figure 4A). At the moment, the clearest views of the molecular mechanism of HJ processing are available from structural and functional data on bacterial RuvA, RuvB, and RuvC and their complexes, including DNA. These results have been summarized in a recent review article.[88]

RuvA forms a fourfold symmetric tetramer to recognize the Holliday junction structure of DNA[89] (Figure 4B). Each subunit consists of distinct domains, domains I, II, and III, among which I and II mainly recognize the Holliday junction structure and III plays a regulatory role in ATP-dependent branch migration through direct contact with RuvB.[90,91] Two forms of the RuvA−Holliday junction DNA complex have been crystallized to date. The crystal structure containing a single RuvA tetramer and the HJ complex has been solved using the *E. coli* RuvA protein.[92,93] On the other hand, the crystal containing two RuvA tetramers, bound to both sides of the junction, has been obtained with *Mycobacterium leprae* RuvA protein.[94] These results suggest that the RuvA tetramers bind to the HJ cooperatively. In these complexes, the junction DNA adopts an almost square planar structure, and each DNA arm assumes a canonical B-form structure. These arms are recognized on the minor groove side by the two helix−hairpin−helix motifs in domain II, using direct and water-mediated hydrogen bonds. The central acidic pin of the RuvA tetramer, formed by Glu55 and Asp56 from each subunit, repels the DNA backbone away from the junction center by electrostatic repulsion and thereby disrupts the two base pairs at the junction center (Figure 4B).

RuvB is a helicase that moves the junction point of HJ and changes the base pairs of the homologous strands. However, the amino acid sequence of the RuvB protein does not share any similarity to those of the helicase superfamily proteins, but it revealed that RuvB is a member of the AAA^+ (ATPase associated with various cellular activities) family of proteins.[95] The crystal structure showed that the RuvB subunit is composed of three domains [N (N-terminal), M (middle), and C (C-terminal) domains] and forms a crescentlike architecture, which is indeed found among the AAA^+ family proteins (Figure 4C). The N and M domains are involved in ATP binding and hydrolysis. Domain N binds domain III of RuvA through its protruding hydrophobic β-hairpin. Domain C has a winged-helix fold, a motif that is frequently involved in DNA binding and is important for pumping out DNA duplexes.[96,97]

The crystal structure of the RuvA−RuvB complex revealed the atomic view of the RuvA−RuvB interaction. In combination with the EM image of the RuvA−RuvB−Holliday junction ternary complex, this structural analysis presented the most convincing atomic structure of the branch migration complex,[98] in which the two oppositely oriented RuvB hexamer ring motors are proposed to pull the DNA duplex arms on both sides of the RuvA bound at the junction center (Figure 4D). Conceivably, the upper and lower acidic pins from each RuvA tetramer promote the exchange of oncoming base pairs at the junction center. The two RuvB hexameric rings are responsible for exerting a spiral rotation force on each encircled DNA arm, using the energy of ATP hydrolysis. The asymmetric contacts of the junction DNA with the two RuvA tetramers suggest that the junction DNA might oscillate between the two RuvA tetramers during branch migration.

RuvC is the endonuclease that cleaves the junction point symmetrically to resolve the HJ and to produce a pair of nicked duplex DNAs.[99,100] RuvC is the first HJ resolvase for which a crystal structure has been determined[101] (Figure 5). Structural analyses combined with mutational studies also provided insights into the reaction mechanism of HJ resolution. The two subunits of the RuvC dimer are related by a dyad axis, and each subunit contains a large cleft, which accommodates the dsDNA. At the bottom of the cleft, the dsDNA contacts the catalytic center, which consists of four acidic residues, Asp7, Glu66, Asp138, and Asp141.[102] The combination of structural and biochemical analyses led to the complex model of RucC−HJ DNA, where the Holliday junction adopts the stacked-X form, rather than the square planar form. The two continuous strands could be simultaneously and symmetrically cleaved near the junction center.[103]

The formation of a RuvABC resolvasome was proposed, based on the physical interaction of these proteins by coimmunoprecipitations.[104] In fact, the junction cleavage by RuvC was facilitated by the presence of RuvA and RuvB.[105] It is still not known how the octameric RuvA complex, which protects the junction from RuvC cleavage, accesses RuvC to cleave the junction DNA. Thus, an atomic view of the RuvABC resolvasome is critical to clarify this problem.

RuvABC could be involved in the stalled replication fork repair by cleaving of the "chicken foot" DNA, which is formed by the unwinding of the stalled replication fork. RuvC can cleave the junction symmetrically, and thus, it collapses the fork to provide a chance to start the following recombination for the replication fork restart. Cleavage of the fork

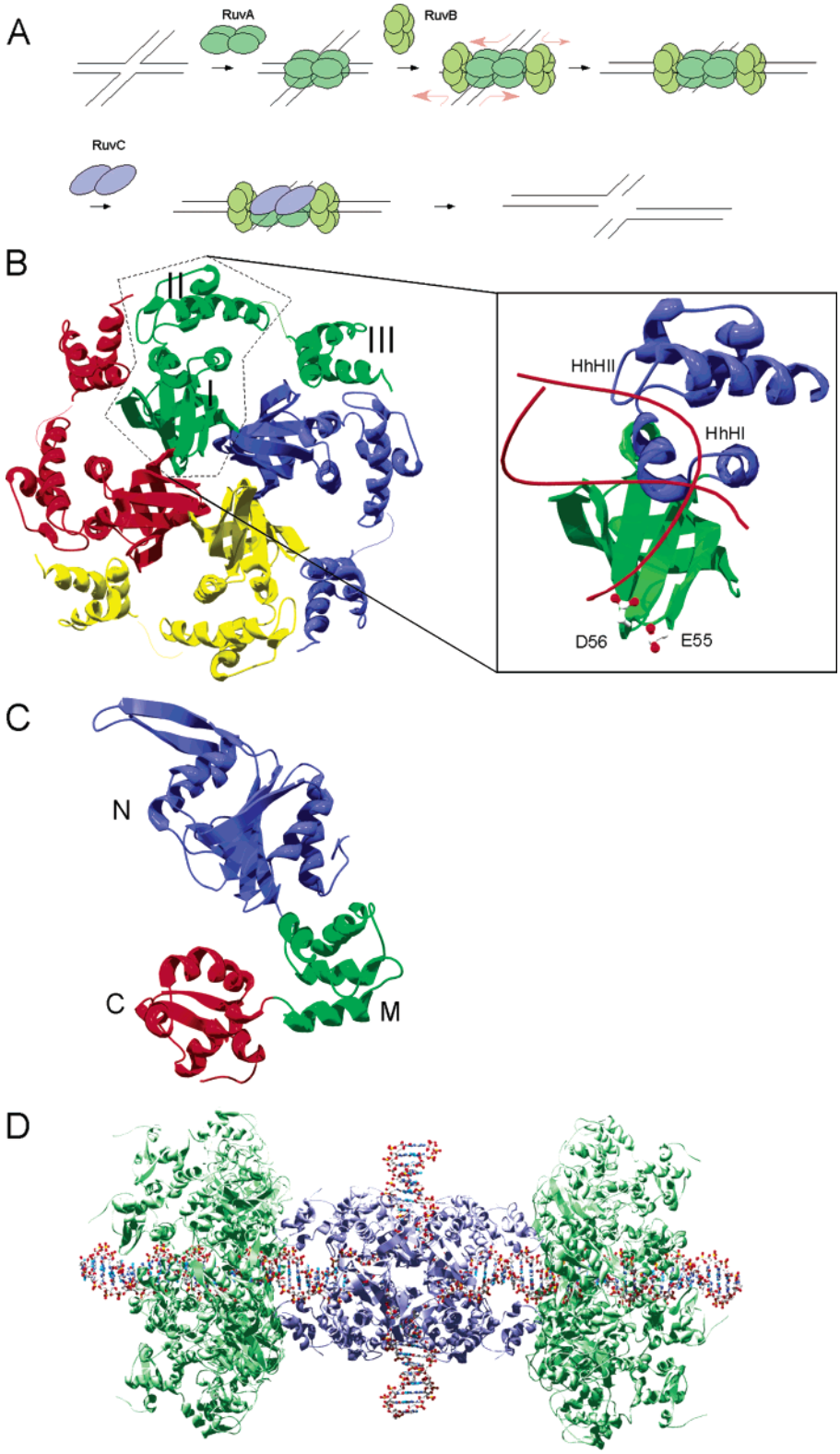

Figure 4. Holliday junction resolution by the bacterial RuvABC resolvasome. (A) Schematic diagram of Holliday junction processing by the RuvABC proteins. RuvA binds to the Holliday junction and recruits RuvB. The RuvAB complex moves the junction utilizing the energy from ATP hydrolysis. It remains unclear how the octameric RuvA complex could be transformed into a state that allows junction resolution by the RuvC dimer. The RuvC protein may replace one of the RuvA tetramers and cleaves the junction after branch migration. (B) Structure of the RuvA tetramer. Each subunit is colored differently. The three structural domains within the RuvA subunit are numbered. (right inset) Crystal structure of the RuvA−Holliday junction. Only domain I (green), domain II (blue), and one arm of the bound DNA are shown. E55 and D56 form an acidic pin, which plays a critical role in junction separation. The bound DNA is shown as a red ribbon. Two strands of DNA are each recognized by the HhH motifs of domain II. (C) Structure of the RuvB monomer. The three domains (N, M, and C) are colored blue, green, and red, respectively. The β-hairpin, which protrudes from the N domain, binds to domain III of RuvA through a hydrophobic interaction. (D) An atomic structure model of RuvAB−HJ DNA based on the crystal structure analyses of RuvA and RuvB combined with the EM image of the RuvA−RuvB−HJ complex.

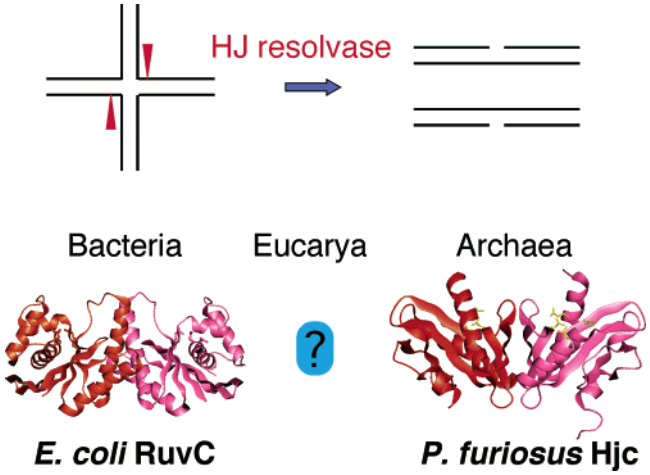

Figure 5. HJ resolvases in cells. RuvC, Hjc, and more? Crystal structures of the bacterial RuvC and the archaeal Hjc are shown. The two subunits are related by twofold symmetry in both of these enzymes, as shown in different colors. The active site residues are displayed as ball-and-stick models.

is especially critical to restart replication when lesions affect both strands of the template DNA.

2.5. RecG Helicase

Genetic analyses revealed that *recG* and *ruv* are involved in the processing of HJ intermediates.[106] RecG is a superfamily 2 (SF2) helicase originally found in *E. coli*, and it can cause Holliday junctions to migrate prior to their resolution by the RuvC resolvase.[107] In *E. coli* cells, the *recG* and *ruv* mutations are strongly synergistic and greatly enhance the sensitivity to UV light, indicating that RecG and RuvABC use different means to process damaged DNA.[108] RecG is able to convert the fork junction structure to the HJ by regression of the junction point at a stalled fork, in an *in vitro* replication system.[109,110] To form a chicken foot structure at a stalled fork, simultaneous unwinding of two duplex DNA strands, both the leading and lagging strands, is required. RecG can perform as a monomer protein to move along both the leading and lagging strand templates to unwind daughter duplexes.[20] To understand the functional mechanism of the RecG helicase from a structural viewpoint, the crystal structure of *Thermotoga maritima* RecG was solved in complex with a synthetic three-way junction, which is a model of the replication fork stalled in the leading strand.[111] This structure showed how the RecG protein recognizes the junction structure, and thus, a mechanism for fork processing to facilitate replication fork restart has been proposed. RecG consists of three domains, 1, 2, and 3, among which domains 2 and 3 form a typical helicase structure with the characteristic motif for SF2 helicases. The interaction site between the protein and the DNA junction is located primarily on domain 1, which is found only in RecG homologues.[112] A significant feature of this domain is the one called the "wedge domain", which forms a significant part of the surface that contacts the bound DNA. RecG binds to the junction, with the arms of the fork arranged around the wedge domain. Only the template strands for the leading and lagging synthesis, but not duplex DNA, would be able to pass through the grooves on either side of the wedge domain. Domains 2 and 3 change their conformation and translocate on the duplex DNA in an ATP-dependent manner, and consequently, the nascent DNA strands would be stripped off the template strands by the simple steric hindrance of the wedge domain. The proximity of the two displaced strands would allow annealing with their complementary sequences. Thus, the function of RecG as a fork reversal machine can be explained by its structural characteristics, as described above.

RecG has some substrate preference for DNA forks containing a duplex in the lagging strand synthesis but not in the leading strand at the branch point *in vitro*.[113] RecG might have a specific role for the conversion of the fork structure, where lagging strand synthesis proceeds beyond a block in leading strand synthesis. Recent biochemical analyses of *E. coli* RecG showed that the specific binding of RecG depends on the full-length protein, although the wedge domain itself is critical for the DNA binding.[114] In addition, the strong binding of the wedge to the branch point also contributes to the processivity of RecG translocation on the dsDNA.[114]

Once the chicken foot has been formed at the stalled fork, DNA synthesis can bypass the lesion by template switching and subsequent regression of the four-way junction to recover the fork structure, or by the cleavage pathway with RuvABC, as described above. RuvABC and RecG are bacterial proteins, and there are no structural (amino acid sequence) homologues in Eukaryotes and Archaea. However, the important role of these proteins in the replication fork repair suggests that a protein factor with RuvABC-like or RecG-like functions should exist in other organisms.

2.6. HJ Processing in Other Organisms

In contrast to the RecA family proteins, HJ processing proteins are not well conserved in the three biological domains. RuvABC homologues exist in a wide variety of bacterial species, although not all bacteria encoding RuvAB also have RuvC.[115] A sequence similarity search failed to detect eukaryotic RuvABC homologues. However, both the junction point migration and cleavage activities have been observed and partially purified from mammalian cell-free extracts.[116−119] The successful identification of the proteins truly responsible for these activities has been anticipated for many years. A recent report showed that RAD51C, a paralogue in human cells, is involved in HJ processing.[120]

Hjc is the archaeal Holliday junction resolvase, which we originally discovered in *P. furiosus*.[37] Using a synthetic HJ as a substrate, we searched for the activity to produce nicked duplex DNAs by symmetrical cleavage of the HJ in the *P. furiosus* cell extract *in vitro*. The target activity was successfully detected after many trials under various conditions. Finally, we identified a new gene encoding a novel HJ resolvase, designated as Hjc. Hjc, as a HJ resolvase, has distinct substrate specificity for HJ cleavage, and the produced nicked duplexes can be rejoined by DNA ligase.[121] Although no supporting genetic data are available thus far, Hjc resolves the RecA-mediated HJ intermediate made by plasmid DNA *in vitro*. These properties of Hjc fulfill the requirements for the HJ resolvase. *P. furiosus* Hjc interacts with RadB, the second RecA/Rad51 family protein in Archaea.[32,35] RadB stimulates the HJ cleavage activity in an ATP-dependent manner,[32] and therefore, the concrete functions of RadB in archaeal cells should be elucidated. The gene encoding Hjc is highly conserved in the archaeal genomes. However, no significant similarity in the amino acid sequences exists between the Hjc and bacterial RuvCs. Furthermore, no sequence homologue of Hjc has been found in eukaryotic genomes.

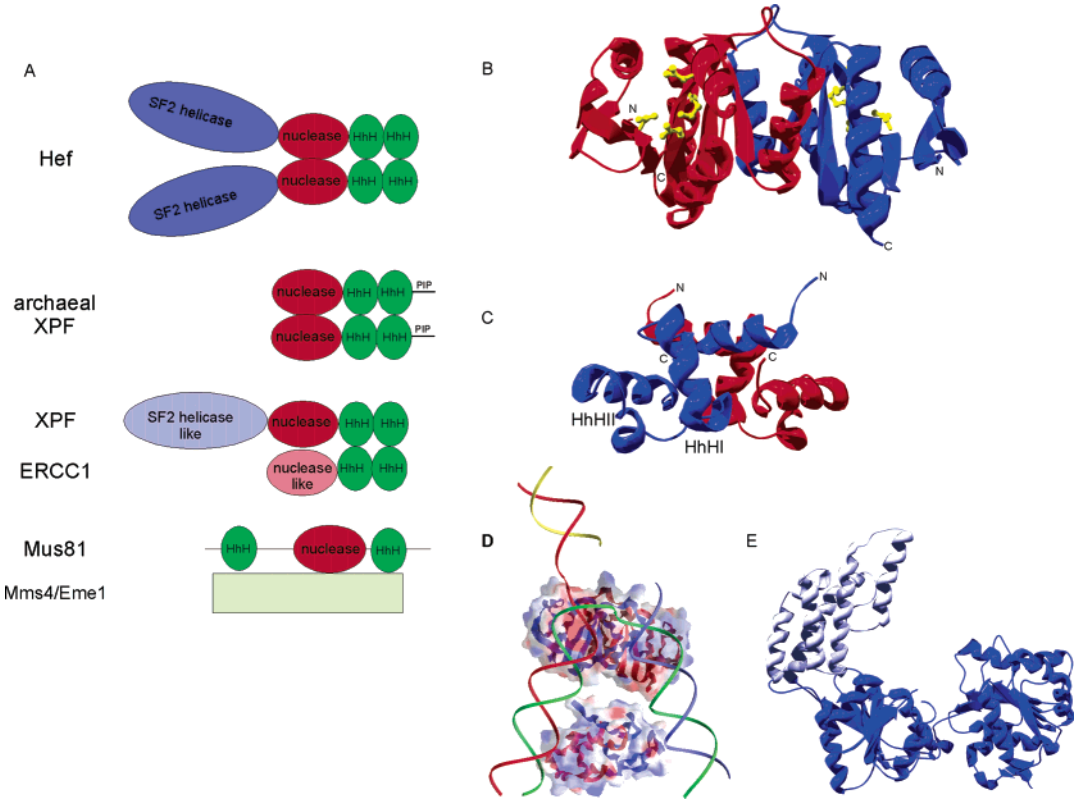

Figure 6. XPF/Mus81/Hef, the structure-specific endonucleases. (A) The domain structures of the XPF/Mus81/Hef family proteins are shown schematically. XPF forms a heterodimer with ERCC1, and Mus81 forms a heterodimer with Eme1/Mms4. Hef is a homodimer. (B) Structure of the Hef nuclease domain dimer. The two subunits are colored red and blue. Active site residues are shown in yellow. (C) Structure of the Hef HhH domain dimer. The two subunits are colored differently, as in part B. The two HhH motifs in a single subunit are labeled. (D) Model of the Hef nuclease with fork-structured DNA. The model is based on the DNA footprinting and base pairing analyses.[139] Each DNA strand is shown as a backbone ribbon. The two HhH domains each bind to the distal arms of the fork DNA. The nuclease domain binds to the junction center. (E) Structure of the Hef helicase domain. The two conserved helicase domains (blue) contain all of the helicase motifs required for ATP hydrolysis and DNA translocation to function as a molecular motor. The insertion domain (light blue) determines the substrate specificity. In the case of Hef, it plays a key role in binding to fork-structured DNA.

The crystal structure of *P. furiosus* Hjc revealed that the folding of the Hjc subunit is vastly different from those of the other HJ resolvases determined thus far, although the homodimer formation is shared as a common feature[39] (Figure 5). However, it is quite interesting that the folding of the region around the active site in Hjc is very similar to those of the type II restriction endonucleases. The configurations of the active site residues are also equivalent to those of restriction endonucleases. In fact, careful sequence analyses revealed a weak similarity between the archaeal Hjc's and several restriction endonucleases, in the region including the active site residues.[122] This observation is intriguing, in terms of the molecular evolution of the HJ resolvase in living organisms. Structural analyses of the HJ resolvase provided the idea that the HJ resolution function has evolved independently from at least four distinct structural folds.[123,124] RuvC is similar to RNaseH, bacteriophage Mu transposase, and HIV integrase, which all share the RNase H fold. On the other hand, Hjc has a fold similar to those of the type I, II, and III restriction endonucleases, MutH, and λ exonuclease. These two groups are now called the integrase superfamily and the nuclease superfamily, respectively. RusA and T4 endonuclease VII do not belong to either of these superfamilies.

It would be difficult to predict a HJ resolvase in eukaryotic cells from the protein structure. The Mus81 family nucleases (described below) may contribute toward resolving the meiotic intermediate to generate crossover products. On the other hand, the RecQ-like helicase (described below) and the type I topoisomerase may achieve HJ resolution to noncrossover products topologically. Current interest is now focused on whether eukaryotic cells have a conventional HJ resolvase with the same characteristics as those of RuvC and Hjc.[27]

3. Other Proteins for Converting Fork-Structured DNA

3.1. Mus81 Complex as a Structure-Specific Endonuclease

The Mus81 protein has been detected in all eukaryotes examined, and it shares a common sequence motif with XPF and Rad1 endonuclease, which are involved in NER. Mus81 associates with Mms4 in *S. cerevisiae* and Eme1 in *S. pombe* and human, which is the same situation as that for XPF−ERCC1 and Rad1−Rad10, and these heterodimers function as DNA structure-specific endonucleases[126−132] (Figure 6A). The Mus81 complex has recently been in the spotlight in the research field of DNA repair and recombination, and there have been lively debates about its *in vivo* function.[27,133] Yeast strains with mutations in *mus81* are sensitive to methylmethane sulfonate and camptothecin, which can stall replication forks, but not to ionizing radiation and bleomycin, which introduce DNA double-strand breaks.[134−137] Recent biochemical analyses on the substrate specificity of the

purified Mus81 complex revealed that this endonuclease directly cleaves stalled replication forks near the junction point, to facilitate the resumption of fork progression after recombination.[128−131] However, the human and yeast Mus81−Eme1 complexes also cleave HJs *in vitro*, and the yeast protein has substrate preference for a nicked HJ.[138,139] It is now proposed that the resolution of the intermediate in meiotic recombination to generate crossovers is one of the functions of the Mus81 nuclease, as described above.[140] In this situation, structural and functional analyses of the Mus81 family proteins are now a very hot research area.

3.2. Hef Protein as a Helicase/Nuclease

After we identified Hjc in archaeal cells, we continued to focus our efforts on identifying the additional HJ resolution−stimulating protein factors. During this course of experiments, we happened to discover a new endonuclease from *P. furiosus*. The results of the screening reaction showed the product generated by the new endonuclease, but no increase in the HJ resolution product (nicked duplex), from the HJ substrate. The new enzyme recognizes a nick within the DNA duplex and cleaves near the nicked site. Further characterization revealed that the new nuclease recognizes branched DNA structures, including a replication forklike structure. The amino acid sequence deduced from the cloned gene showed that the enzyme is composed of two distinct regions. The amino-terminal two-thirds encodes a helicase-like sequence, which is classified into the SF2 helicases, and the remaining C-terminal one-third exhibits similarity to nuclease family proteins, including XPF and Rad1 (Figure 6A). From these observations, we designated this novel protein as Hef, according to initials of the *H*elicase-associated *e*ndonuclease for *f*ork structure.[40] We were unable to overproduce the recombinant Hef as the full-length protein in *E. coli*. However, we found that the two regions can be separately overproduced, and thus, each region was used for structural and functional analyses. Both the helicase and nuclease regions have specific affinity for branched DNA structures.[40] When we found that the nuclease domain of Hef can cleave the fork-structured DNA near the junction point *in vitro*, three reports, describing the endonuclease activity of Mus81, were coincidentally published. On the basis of the substrate specificity, it was proposed that *S. pombe* and human Mus81 can be assigned to nuclear HJ resolvases in eukaryotic cells.[125,126] On the other hand, *S. cerevisiae* Mus81 was presented as an endonuclease for stalled replication forks, but not for the HJ.[127] The substrate specificity of the archaeal Hef supports the function of the eukaryotic Mus81 as an endonuclease that cleaves the stalled fork directly at the branch point.

Analogous to the dimer formation of XPF and Rad1 with ERCC1 and Rad10, respectively, Mus 81 also forms a dimer with Mms4 or Eme1, as described above. In contrast to these eukaryotic proteins, Hef works as a homodimeric form (Figure 6A). The dimer formation seems to be essential for the expression of the endonuclease activity in this family of proteins. The endonuclease region of Hef can be divided into two domains, the endonuclease catalytic domain and the HhH domain, by a partial proteolysis. Both of the domains contribute to dimer formation.[141] The crystal structure of the endonuclease catalytic domain (Figure 6B) showed that the folding, particularly in the vicinity of the sequence motif of GDXnERKX3D, has remarkable similarity to those of restriction endonucleases.[141] On the other hand, the dimer interface, which is completely different from those of restriction endonucleases, showed strong conservation with those of the XPF/Rad1/Mus81 family proteins. The dimer interfaces are formed by two regions: one in the nuclease catalytic domain and the other in the HhH domain, respectively (Figure 6B and C). Interestingly, both interfaces have to be disrupted to generate a monomer mutant of Hef, which lacks the nuclease activity for the fork-structured DNA.[141] Our biochemical and structural analyses predicted that each of the endonuclease and HhH domains of Hef binds near the junction center of the forked DNA.[142] On the basis of these findings, a model structure of the Hef nuclease−fork DNA complex was built (Figure 6D). In this model, individual HhH domains from two separate subunits asymmetrically bind to the arm region, while the endonuclease domain binds near the junction center, where the base pairs are disrupted for cleavage upon Hef binding. The crystal structure of the Hef endonuclease homologue from a different archaeon, *Aeropyrum pernix*, has recently been published.[143] Comparison of the crystal structures between the protein alone and the dsDNA-bound form revealed a large domain movement upon DNA binding. Identification of the two nonequivalent DNA binding sites, in addition to the above domain movement, allowed the prediction of a molecular mechanism for how this family of nucleases recognizes and cleaves DNA with specific structures. Considering the sequence conservation and the above functional and structural aspects, including the domain arrangement and the dimeric nature, Hef is likely to be ancestral to its eukaryotic homologues, XPF, Rad1, and Mus81.

The Hef helicase region at the N-terminus indeed possesses a helicase activity for DNA with the fork structure. Its ATPase activity is also remarkably stimulated by the addition of fork-structured DNA. The Hef nuclease alone acts poorly on a DNA fork containing single-stranded gaps, whereas the Hef helicase alone works efficiently on gap-containing forked DNA. Thus, the endonuclease works cooperatively with the helicase, which can modify the DNA substrate so that it assumes the fork structure most favorable for cleavage.[41] The crystal structure of the helicase domain of *P. furiosus* Hef revealed a novel helicase insertion between the two conserved helicase core domains (Figure 6E). This inserted domain, which is similar to the "thumb" domain of DNA polymerase, is positively charged and plays critical roles in fork recognition.[144] The Hef helicase shares sequence similarity with *S. cerevisiae* Mph1, which was originally identified by virtue of the spontaneous mutator phenotype of the deletion mutants of its corresponding *MPH1* gene.[145] Further genetic analyses revealed epistasis of the *mph1* with mutations in the *RAD52* group that mediate homologous recombination. The mutator phenotype of *mph1* depends on REV3 and REV1, and is synergistic with mutation in genes involved in excision repair. These results led to the proposal that the Mph1 protein is involved in an error-free DNA damage bypass pathway.[146] The purified Mph1 protein has an ATP-dependent DNA helicase activity, which is enhanced in the presence of RPA.[147] The human genome contains a gene encoding a Hef homologue, which should be analyzed urgently from both the enzymatic and structural points of view.

During the preparation of this manuscript, two reports were published demonstrating that the human Hef homologue functions as a member of a replication fork repair pathway, which is related to Fanconi anemia (FA), a famous genetic

disease caused by genome instability.[43,44] This discovery sheds light on the elucidation of the pathologic mechanism of FA, and the function of Hef in this repair pathway is now a noticeable issue.

3.3. RecQ Helicase Family

The two RecA-dependent DNA repair pathways in Bacteria are important for the repair of stalled or collapsed DNA replication forks.[10] The two pathways, the RecBCD pathway and the RecF pathway, probably complement each other for some of the functions.[148] Many proteins, including RecA, RecF, RecG, RecJ, RecN, RecO, RecQ, and RecR, are involved in the RecF pathway, and these proteins are known to be required for the repair of gapped DNA and for the resumption of DNA synthesis after UV-induced DNA damage.[149−151] The RecF pathway is also important to stabilize the nascent strands at stalled forks, because, in the absence of RecF protein, the nascent lagging strand of the arrested replication fork is extensively degraded by the RecJ nuclease after unwinding by the RecQ helicase.[23,24]

RecQ was originally discovered in *E. coli* as a recombination protein involved in the RecF pathway,[152] and it catalyzes reactions that help to repair the stalled replication forks. The *E. coli* RecQ protein bears the activities for not only ATP-dependent DNA unwinding but also recombination initiation with RecA and SSB. Three genetic diseases, Bloom's, Werner's, and Rothmund−Thomson syndromes, are now well-known to be related to the mutations of human genes, *BML*, *WRN*, and *RecQ4*, which encode proteins homologous to RecQ.[25,26] A common feature of these diseases is the enhanced genome instability, which is manifested by high levels of homologous recombination and chromosomal deletions, resulting in a predisposition to cancer. These facts highlight the important function of the RecQ family proteins for the maintenance of genome stability in the cells.

Amino acid sequence analyses indicated that the *E. coli* RecQ protein has three conserved regions, the helicase, RecQ-conserved (RecQ-Ct), and HRDC (helicase and RNaseD-C-terminal) regions.[153] The helicase and RecQ-Ct regions form the catalytic core, RecQ, and fold together to form a single 59 kDa domain, RecQΔC. RecQΔC has the DNA-dependent ATPase and DNA unwinding activities with the same specific activities as the wild-type RecQ.[154] The crystal structure has been determined for RecQΔC, which lacks the C-terminal HRDC region.[155] RecQΔC comprises four subregions, of which two are conserved helicase regions and the other two combine and form a single domain, RecQ-Ct. Two DNA binding sites were predicted in the structure of RecQ. A ssDNA binding site was predicted from the structural similarity in comparison with other helicases. A winged helix (WH) motif, containing a helix−turn−helix fold, exists in the RecQ-Ct domain, and a large cleft resides at the intersection of the WH and the adjacent Zn^{2+} binding helical subdomain. This cleft seems to serve as a binding site for dsDNA. These structural data allowed the construction of a model of the RecQ−DNA complex, in which the 3′-ssDNA end of the unwound DNA is bound to the helicase region. It is interesting that the seven missense mutations in the human *BLM* gene that cause Bloom's syndrome were mapped to the RecQ family catalytic core domain, with five of them occurring in residues identically conserved within the *E. coli* RecQ protein. The enzymatic activities of the RecQ family proteins should be important to prevent serious diseases. However, further studies are essential to understand the substrate specificity and the molecular mechanism of the RecQ helicase activity. Especially, it is still unknown whether RecQ works as a monomer or an oligomer in the cells. A number of inconsistent results have been reported, in terms of the active oligomeric structures.[156−160] The major subdomain of RecQ is structurally homologous to the helicase and WH regions of the reverse gyrase from *Archaeoglobus fulgidus*,[161] and therefore, this structural similarity may reveal the functional similarity of the RecQ-associated topoisomerase III activity to that of the reverse gyrase.

3.4. RecJ Exonuclease

The RecJ protein has a ssDNA-specific 5′-exonuclease activity, which degrades the nascent lagging strand after it is unwound by the RecQ helicase.[23,162] The crystal structure of RecJ from *Thermus thermophilus* HB8 bound to a Mn^{2+} ion revealed a unique fold, in which the two domains are interconnected by a long helix.[163] There is a groove at the center, and its wall has an abundance of positive charges from basic residues. The Mn^{2+} ion is also located on the wall and is coordinated by residues conserved in the phosphoesterase family. The narrow width of the groove indicates that RecJ specifically binds ssDNA, but not dsDNA.

3.5. Hjm Structure-Specific Helicase

The mechanism for HJ migration in archaeal cells has been an open question, and thus, we searched for a branch migration activity corresponding to that displayed by the bacterial RuvAB proteins. As a result, we have successfully identified a new helicase that unwinds a synthetic fourway junction *in vitro*, from a *P. furiosus* cell extract. The cloning of the gene encoding this activity revealed that the deduced amino acid sequence has similarity to those of the SF2 helicases. The purified recombinant protein can specifically dissociate a synthetic HJ and a plasmid-based recombination intermediate (α-structure) produced by the RecA protein *in vitro*, and thus, we designated it as Hjm (Holliday junction migration).[164] Further characterizations showed that Hjm preferably binds and unwinds the strands from the forklike structures. In addition, some genetic studies suggested that Hjm is a functional homologue of the RecQ protein in Archaea.[165] A sequence homology search revealed some similarity between Hjm and eukaryotic Mus308, Hel308, and Pol Q. The *D. melanogaster mus308* mutant is sensitive to cross-linking agents, such as psoralen, diepoxybutane, and nitrogen mustard, and therefore, the gene product seems to function in interstrand cross-link (ICL) repair, from genetic studies.[166] The Mus308 protein has a family A DNA polymerase (bacterial DNA polymerase I)-like sequence at the C-terminal region, in addition to the N-terminal SF2 helicase sequence.[166] Moreover, this protein probably has DNA polymerase activity.[167] The human Hel308, isolated as a homologue of Mus308, does not have the DNA polymerase-like sequence. The Hel308 protein has been purified from recombinant insect cells, and activities including a single-stranded DNA-dependent ATPase and a DNA helicase, which translocates on DNA with 3′ to 5′ polarity, were demonstrated using the purified protein.[168] Human Pol Q, which has a bacterial Pol I-like sequence,[169] has been identified recently to have a helicase-like sequence at the N-terminal region.[170] Therefore, Pol Q is entirely similar to Mus308, and these two genes seem to be orthogonous. Concrete functions of these eukaryotic proteins should be

clarified. The archaeal protein, isolated from *Methanothermobactor thermoautotrophicus* as a homologue of Hel308 and named as Hel308a, also has RecQ-like functions exactly the same as that of *P. furiosus* Hjm.[171] The functional interaction (stimulation of the helicase activity) of Hjm with PCNA is also consistent with the previous reports showing the direct interactions between WRN (a RecQ family protein) and PCNA from mouse[172] and human.[173,174] The crystal structure of Hjm showed five distinct domains, in which domains I and II form the conserved RecA-like helicase fold and domains III−V adopted a unique structure that is probably involved in recognizing a specific DNA structure (Oyama et al., unpublished).

3.6. Recombination Mediator Proteins, RecFOR and Rad52

Among the RecF pathway proteins, the RecF protein forms a stable complex with RecO and RecR, and this complex protects the nascent lagging strand of the arrested replication forks to maintain the fork structure.[175−177] This RecFOR complex is recognized as a member of the RMPs (recombination/replication mediator proteins, a specialized class of SSBs), and they promote enzyme−ssDNA assembly by overcoming the inhibition exerted by the conventional SSB.[178] In addition, the RecFOR complex loads RecA onto gapped DNA that is coated with SSB, thereby accelerating DNA strand exchange.[179] To understand the concrete function of the RecFOR complex, the crystal structures of RecR[180] and RecO[181] from *D. radiodurans*, which is highly resistant to ionizing radiation and lacks RecBCD homologues, have been determined.

RecR consists of an N-terminal domain, with a HhH motif, and a C-terminal domain, which contains Cys4-zinc-finger, Toprim, and Walker B motifs, respectively. The RecR subunits form a ring-shaped tetramer with a central 30−35 Å diameter hole. The overall shape of the RecR tetramer is strikingly similar to the ring-shaped sliding clamps including the archaeal and eukaryotic PCNAs and the bacterial β-subunit of Pol III, despite the lack of similarity in the primary sequence and the quaternary structure. It has been proposed that RecR, in complex with RecF or RecO, may function as a nonsliding clamp that recognizes the structural features of ssDNA−dsDNA junctions.

The RecO protein consists of three domains. The N-terminal OB domain, which is very similar to those of RPA and SSB, is connected to a three-helix bundle followed by the Cys4-zinc-finger motif. The tight packing of the central helical bundle may play a structural role, by maintaining the OB fold and the zinc-finger motif in their respective positions for appropriate DNA binding. The overall similarity to the heterotrimeric core of RPA suggests that RecO may be evolutionally related to the eukaryotic ssDNA binding protein. The conserved aromatic residues in the OB folds of ssDNA binding proteins are replaced by positively charged residues in RecO. This suggests a modified DNA binding mode, which may explain the strand annealing activity of RecO.

RecO and RecR form a heterohexamer complex with a 2 to 4 ratio. Each protein has binding activity to both ssDNA and dsDNA. However, the specific binding of the RecFOR complex to the ssDNA−dsDNA junction in a stalled replication fork indicates that complex formation is necessary to ensure the structure-specific recognition.

The biochemical properties of Rad52 imply that it may be a functional homologue of RecO, which facilitates the displacement of RPA by Rad51 and also anneals RPA-coated ssDNA with its complementary ssDNA in eukaryotic cells.[178,182,183] The crystal structure of the N-terminal part of Rad52 revealed an undecameric subunit ring and a positively charged groove running along the surface of the ring.[184,185] These structural properties are similar to those of RecO. The intact Rad52 protein is known to form a heptameric ring for catalyzing homologous pairing.[186] These inconsistent oligomerizations between Rad52 and the N-terminal domain suggest that Rad52 alters its conformation in response to distinct functions. The heptameric and undecameric rings may be more suitable for transferring DNA to Rad51 and for performing homologous pairing, respectively.

4. Restart of Replication

Reloading of the replication machinery at damaged fork sites is required to retrieve the arrested fork and to restart replication. In Bacteria, the PriA protein plays a key role in restarting replication, as a sensor for the state of fork progression.[187,188] PriA can recognize and bind to the 3′-termini of arrested forks and recruits the replicative DnaB helicase and DnaG primase.[189,190] The PriA protein has helicase and DNA-dependent ATPase activities. A limited proteolytic analysis revealed that the 181 amino acid N-terminal domain can form a complex with D-loop structured DNA.[191] Recent structural analyses have facilitated the identification of the critical residues for the recognition of the 3′-terminus of the DNA and have led to the proposal that a 3′-terminal binding pocket exists in the PriA protein.[192] The ability of the PriA protein to specifically recognize the 3′-terminus of a hybridizing DNA strand would be suitable for a stalled fork sensor. It is not known whether an analogous system for replication restart exists in eukaryotic cells. It would be interesting to identify a eukaryotic protein that recognizes the 3′-end of the DNA strand.

5. Concluding Remarks and Future Outlook

It was believed that homologous recombination mainly functions for the generation of genetic diversity, but now it is also recognized as a crucial repair system for the maintenance of genome integrity, especially by restoring stalled forks during DNA replication. Studies on the molecular mechanisms of homologous recombination in *E. coli* have been fruitful, and various protein factors involved in the process have been identified. On the other hand, the identification of Rad51 in eukaryotic cells has promoted molecular biology of eukaryotic homologous recombination, resulting in the accumulation of genetic and biochemical data from yeast and mammalian cells. On the basis of these biochemical and genetic data, several models have been proposed for replication fork repair at stalled sites.[193−195] In comparison with the case of prokaryotes, obviously more complicated mechanisms, involving many protein factors, work in eukaryotic cells. Here, we have described the structural and functional aspects of many important proteins, most of which are involved in recombinational repair processes in Bacteria and Archaea. In general, DNA processing proteins contain multiple structural domains, each with a distinct function. Furthermore, these proteins contain versatile structural modules to interact with DNA and other partner proteins. These data have provided detailed insights into how the proteins recognize and process DNA with irregular and complex structures during recombinational

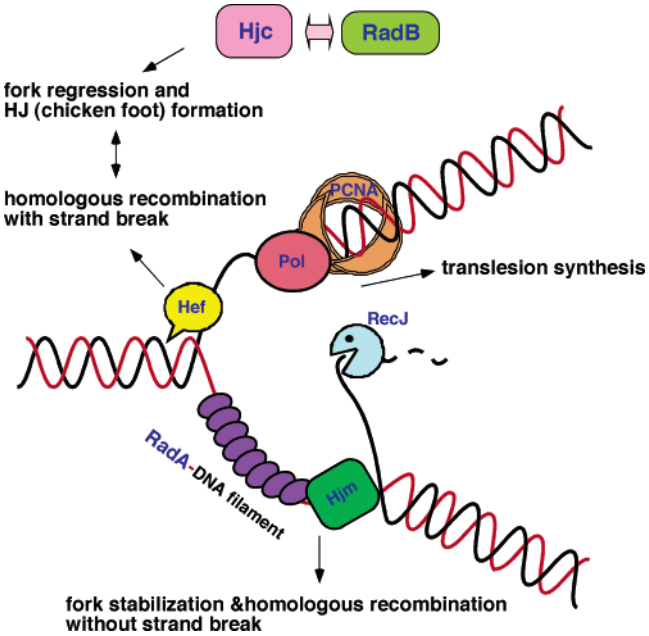

Figure 7. Repair system for stalled replication forks. The stalled replication fork may be systematically restored by many protein factors to maintain genome integrity. Archaeal proteins involved in this repair system are shown here. Hef may cleave the junction point of the fork to lead a homologous recombination repair pathway. Hjc may function when a four-way junction is formed by fork regression, and RadB may be a regulator of this process. Hjm may be involved in a homologous recombination repair pathway without strand break. A translesion synthesis pathway in Archaea is not well-known yet. Most of these proteins have already been crystallized and the 3D structures have been determined.

repair. However, despite the abundance of structural and biochemical data for the archaeal proteins presented here, our knowledge of these repair systems is still rather limited, and further studies will be necessary to solve the puzzle of recombinational repair system comprehensively (Figure 7). In the process of DNA replication and repair, many proteins form huge protein−DNA complexes, such as replisomes, and recombinosomes, for example, which have to convert their structures by exchanging component proteins in response to repair system requirements. Therefore, structural analyses of supermolecular complexes, as well as protein complexes including DNA substrates, are necessary to obtain clearer details and insights into the molecular mechanisms of the recombinational repair process. However, many of these complexes are too unstable to be determined by X-ray crystallography. In this context, proteins from hyperthermophilic Archaea or Bacteria should be advantageous for structural determinations because of their enhanced stability. Archaeal proteins are particularly useful targets for structural determinations, since they are generally recognized as good models of eukaryotic proteins in replication and recombinational repair. Efforts to determine each complex structure involved in the multiple processing steps will allow us to understand the fundamental activities of the DNA processing machinery, which is dynamically assembled and disassembled in conjunction with the cell cycle and in response to DNA damage.[196]

6. Acknowledgments

This work was supported in part by a grant from the Human Frontier Science Program (to Y.I. and T.N.), a grant-in-aid from the Ministry of Education, Science, and Sports of Japan (to Y.I.), and the Japan New Energy and Industrial Technology Development Organization (to BERI). We apologize to the researchers whose work was not cited due to space limitations.

7. References

(1) Kunkel, T. A.; Bebenek, K. *Annu. Rev. Biochem.* **2000**, *69*, 497.
(2) Carr, A. M. *DNA Repair* **2002**, *1*, 983.
(3) Sancar, A.; Lindsey-Boltz, L. A.; Unsal-Kaccmaz, K.; Linn, S. *Annu. Rev. Biochem.* **2004**, *73*, 39.
(4) Bierne, H.; Michel, B. *Mol. Microbiol.* **1994**, *13*, 17.
(5) McGlynn, P.; Lloyd, R. G. *Nature Rev. Mol. Cell Biol.* **2003**, *3*, 859.
(6) Hochegger, H.; Sonoda, E.; Takeda, S. *BioEssays* **2004**, *26*, 151.
(7) Lehmann, A. R. *Cell Cycle* **2003**, *2*, 300.
(8) Jansen, J. G.; de Wind, N. *DNA Repair* **2003**, *2*, 1075.
(9) Yang, W. *Curr. Opin. Struct. Biol.* **2003**, *13*, 23.
(10) Cox, M. M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8173.
(11) Michel, B.; Flores, M. J.; Viguera, E.; Grompone, G.; Seigneur, M.; Bidnenko, V. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8181.
(12) McGlynn, P.; Lloyd, R. G. *Trends Genet.* **2002**, *18*, 413.
(13) Cox, M. M. *Mutat. Res.* **2002**, *510*, 107.
(14) Holliday, R. *Genet. Res.* **1964**, *5*, 282.
(15) Higgins, N. P.; Kato, K.; Strauss, B. A. *J. Mol. Biol.* **1976**, *101*, 417.
(16) Sogo, J. M.; Lopez, M.; Foiani, M. *Science* **2000**, *297*, 599.
(17) Briggs, G. S.; Mahdi, A. A.; Weller, G. R.; Wen, Q.; Lloyd, R. G. *Philos. Trans. R. Soc. London B* **2004**, *359*, 49.
(18) Robu, M. E.; Inman, R. B.; Cox, M. M. *J. Biol. Chem.* **2004**, *279*, 10973.
(19) Seigneur, M.; Bidnenko, V.; Ehrlich, S. D.; Michel, B. *Cell* **1998**, *95*, 419.
(20) McGlynn, P.; Lloyd, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8227.
(21) Hamon, F. G.; Kowalczykowski, S. C. *Genes Dev.* **1998**, *12*, 1134.
(22) Hishida, T.; Han, Y. W.; Shibata, T.; Kubota, Y.; Ishino, Y.; Iwasaki, H.; Shinagawa, H. *Genes Dev.* **2004**, *18*, 1886.
(23) Coucelle, J.; Hanawalt, P. C. *Mol. Gen. Genet.* **1999**, *262*, 543.
(24) Coucelle, J.; Crowley, D. J.; Hanawalt, P. C. *J. Bacteriol.* **1999**, *181*, 916.
(25) Bachrati, C.; Hickson, I. D. *Biochem. J.* **2003**, *374*, 577.
(26) Khakhar, R. R.; Cobb, J. A.; Bjergbaek, L.; Hickson, I. D.; Gasser, S. M. *Trends Cell Biol.* **2003**, *13*, 493.
(27) Heyer, W. D.; Ehmsen, K. T.; Solinger, J. A. *Trends Biochem. Sci.* **2003**, *28*, 548.
(28) Haber, J. E.; Heyer, W. D. *Cell* **2001**, *107*, 551.
(29) Woese, C. R.; Fox, G. E. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5088.
(30) Olsen, G. J.; Woese, C. R. *Cell* **1997**, *89*, 991.
(31) Hayashi, I.; Morikawa, K.; Ishino, Y. *Nucleic Acids Res.* **1999**, *27*, 4695.
(32) Komori, K.; Miyata, T.; DiRuggiero, J.; Holley-Shanks, R.; Hayashi, I.; Cann, I. K. O.; Mayanagi, K.; Shinagawa, H.; Ishino, Y. *J. Biol. Chem.* **2000**, *275*, 33782.
(33) Komori, K.; Miyata, T.; Daiyasu, H.; Toh, H.; Shinagawa, H.; Ishino, Y. *J. Biol. Chem.* **2000**, *275*, 33791.
(34) Uemori, T.; Sato, Y.; Kato, I.; Ishino, Y. *Genes Cells* **1995**, *2*, 499.
(35) DiRuggiero, J.; Robb, F. In *New Developments in Marine Bio/Technology*; Le Gal, Y., Halvorson, H., Eds.; Plenum Press: New York, 1998; pp 193−196.
(36) Komori, K.; Ishino, Y. *J. Biol. Chem.* **2001**, *276*, 25654.
(37) Komori, K.; Sakae, S.; Shinagawa, H.; Morikawa, K.; Ishino, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 8873.
(38) Komori, K.; Sakae, S.; Daiyasu, H.; Toh, H.; Morikawa, K.; Shinagawa, H.; Ishino, Y. *J. Biol. Chem.* **2000**, *275*, 40385.
(39) Nishino, T.; Komori, K.; Tsuchiya, D.; Ishino, Y.; Morikawa, K. *Structure* **2001**, *9*, 197.
(40) Komori, K.; Fujikane, R.; Shinagawa, H.; Ishino, Y. *Genes Genet. Syst.* **2002**, *77*, 227.
(41) Komori, K.; Hidaka, M.; Horiuchi, T.; Fujikane, R.; Shinagawa, H.; Ishino, Y. *J. Biol. Chem.* **2004**, *279*, 53175.
(42) Roberts, J. A.; Bell, S. D.; White, M. F. *Mol. Microbiol.* **2003**, *48*, 361.
(43) Meetei, A. R.; Medhurst, A. L.; Ling, C.; Singh, T. R.; Bier, P.; Steltenpool, J.; Stone, S.; Dokal, I.; Mathew, C. G.; Hoatlin, M.; de Winter, J. P.; Wang, W. *Nat. Genet.* **2005**, *37*, 958.
(44) Mosedale, G.; Niedzwiedz, W.; Alpi, A.; Perrina, F.; Pereira-Leal, J. B.; Johnson, M.; Langevin, F.; Pace, P.; Patel, K. J. *Nat. Struct. Mol. Biol.* **2005**, *12*, 763−771.
(45) Nishino, T.; Morikawa, K. *Oncogene* **2002**, *21*, 9022.
(46) Shin, D. S.; Chahwan, C.; Huffman, J. L.; Tainer, J. A. *DNA Repair* **2004**, *3*, 863.

(47) Cox, M. M. *Annu. Rev. Microbiol.* **2003**, *57*, 551.
(48) Egelman, E. H.; Stasiak, A. *J. Mol. Biol.* **1986**, *191*, 677.
(49) Yu, X.; Egelman, E. H. *J. Mol. Biol.* **1992**, *227*, 334.
(50) Story, R. M.; Weber, I. T.; Steitz, T. A. *Nature* **1992**, *355*, 318.
(51) Datta, S.; Prabu, M. M.; Vaze, M. B. *Nucleic Acids Res.* **2000**, *28*, 4964.
(52) Datta, S.; Krishna, R.; Ganesh, N.; Chandra, N. R.; Muniyappa, K.; Vijayan, M. *J. Bacteriol.* **2003**, *185*, 4280.
(53) Xing, X.; Bell, C. E. *J. Mol. Biol.* **2004**, *342*, 1471.
(54) Rajan, R.; Bell, C. E. *J. Mol. Biol.* **2004**, *344*, 951.
(55) Yu, X.; Egelman, E. H. *Nature Struct. Biol.* **1997**, *4*, 101.
(56) Conway, A. B.; Lynch, T. W.; Zhang, Y.; Fortin, G. S.; Fung, C. W.; Symington, L. S.; Rice, P. A. *Nature Struct. Mol. Biol.* **2004**, *11*, 791.
(57) Yu, X.; Jacobs, S. A.; West, S. C.; Ogawa, T.; Egelman, E. H. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8419.
(58) Mcllwraith, M. J.; Hall, D. R.; Stasiak, A. Z.; Stasiak, A.; Wigley, D. B.; West, S. C. *Nucleic Acids Res.* **2001**, *29*, 4509.
(59) Yang, S.; Yu, X.; Seitz, E. M. *J. Mol. Biol.* **2001**, *314*, 1077.
(60) Ariza, A.; Richard, D. J.; White, M. F.; Bond, C. S. *Nucleic Acids Res.* **2005**, *33*, 465.
(61) Shin, D. S.; Pellegrini, L.; Daniels, D. S.; Yelent, B.; Craig, L.; Bates, D.; Yu, D. S.; Shivji, M. K.; Hitomi, C.; Arvai, A. S.; et al. *EMBO J.* **2003**, *22*, 4566.
(62) Haruta, N.; Yu, X.; Yang, S.; Egelman, E. H.; Cox, M. M. *J. Biol. Chem.* **2003**, *278*, 52710.
(63) Wu, Y.; He, Y.; Moya, I. A.; Qian, X.; Luo, Y. *Mol. Cell* **2004**, *15*, 423.
(64) Wu, Y.; Qian, X.; He, Y.; Moya, I. A.; Luo, Y. *J. Biol. Chem.* **2005**, *280*, 722.
(65) Kowalczykowski, S. C.; Dixon, D. A.; Eggleston, A. K.; Lauder, S. D.; Rehrauer, W. M. *Microbiol. Rev.* **1994**, *58*, 401.
(66) Boehmer, P. E.; Emmerson, P. T. *Gene* **1991**, *102*, 1.
(67) Handa, N.; Ohashi, S.; Kusano, K.; Kobayashi, I. *Genes Cells* **1997**, *2*, 525.
(68) Dillingham, M. S.; Spies, M.; Kowalczykowski, S. C. *Nature* **2003**, *432*, 893.
(69) Shingleton, M. R.; Dillingham, M. S.; Gaudler, M.; Kowalczykowski, S. C.; Wigley, D. B. *Nature* **2004**, *432*, 187.
(70) Lohman, T. M.; Ferrari, M. E. *Annu. Rev. Biochem.* **1994**, *63*, 527.
(71) Wold, M. S. *Annu. Rev. Biochem.* **1997**, *66*, 61.
(72) Iftode, C.; Daniely, Y.; Borowiec, J. A. *Crit. Rev. Biochem. Mol. Biol.* **1999**, *34*, 141.
(73) Bochkarev, A.; Bochkareva, E. *Curr. Opin. Struct. Biol.* **2004**, *14*, 36.
(74) Sugiyama, T.; Zaitseva, E. M.; Kowalczykowski, S. C. *J. Biol. Chem.* **1997**, *272*, 7940.
(75) Sung, P. *J. Biol. Chem.* **1997**, *272*, 28194.
(76) Bochkarev, A.; Pfuetzner, R. A.; Edwards, A. M.; Frappier, L. *Nature* **1997**, *385*, 176.
(77) Bochkareva, E.; Belegu, V.; Korolev, S.; Bochkarev, A. *EMBO J.* **2001**, *20*, 612.
(78) Raghunathan, S.; Ricard, C. S.; Lohman, T. M.; Waksman, G. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 6652.
(79) Raghunathan, S.; Kozlov, A. G.; Lohman, T. M.; Waksman, G. *Nat. Struct. Biol.* **2000**, *7*, 648.
(80) Bernstein, D. A.; Eggington, J. M.; Killoran, M. P.; Misic, A. M.; Cox, M. M.; Keck, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 8575.
(81) Wadsworth, R. I.; White, M. F. *Nucleic Acids Res.* **2001**, *29*, 914.
(82) Kerr, I. D.; Wadsworth, I. M.; Cubeddu, L.; Blankenfeldt, W.; Naismith, J. H.; White, M. F. *EMBO J.* **2003**, *22*, 2561.
(83) Bochkarev, A.; Bochkareva, E.; Frappier, L.; Edwards, A. M. *EMBO J.* **1999**, *18*, 4498.
(84) Bochkareva, E.; Korolev, S.; Lee-Miller, S. P.; Bochkarev, A. *EMBO J.* **2002**, 21, 1855.
(85) Blackwell, L. J.; Borowiec, J. A.; Masrangelo, I. A. *Mol. Cell. Biol.* **1996**, *16*, 4798.
(86) Sinagawa, H.; Iwasaki, H. *Trends Biochem. Sci.* **1996**, *20*, 387.
(87) West, S. C. *Annu. Rev. Genet.* **1997**, *31*, 213.
(88) Yamada, K.; Ariyoshi, M.; Morikawa, K. *Curr. Opin. Struct. Biol.* **2004**, *14*, 130.
(89) Rafferty, J. B.; Sedelnikova, S. E.; Hargreaves, D.; Artymiuk, P. J.; Baker, P. J.; Sharples, G. J.; Mahdi, A. A.; Lloyd, R. G.; Rice, D. W. *Science* **1996**, *274*, 415.
(90) Nishino, T.; Ariyoshi, M.; Iwasaki, H.; Shinagawa, H.; Morikawa, K. *Structure* **1998**, *6*, 11.
(91) Nishino, T.; Iwasaki, H.; Kataoka, M.; Ariyoshi, M.; Fujita, T.; Shinagawa, H.; Morikawa, K. *J. Mol. Biol.* **2000**, *298*, 407.
(92) Hargreaves, D.; Rice, D. W.; Sedelnikova, S. E.; Artymiuk, P. J.; Lloyd, R. G.; Rafferty, J. B. *Nat. Struct. Biol.* **1998**, *5*, 441.
(93) Ariyoshi, M.; Nishino, T.; Iwasaki, H.; Shinagawa, H.; Morikawa, K. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 8257.
(94) Roe, S. M.; Barlow, T.; Brown, T.; Oram, M.; Keeley, A.; Tsaneva, I. R.; Pearl, L. H. *Mol. Cell* **1998**, *2*, 361.
(95) Iwasaki, H.; Han, Y.-H.; Okamoto, T.; Ohnishi, T.; Yoshikawa, M.; Yamada, M.; Toh, H.; Daiyasu, H.; Ogura, T.; Shinagawa, H. *Mol. Microbiol.* **2000**, *19*, 6266.
(96) Yamada, K.; Kunishima, N.; Mayanagi, K.; Ohnishi, T.; Nishino, T.; Iwasaki, H.; Shinagawa, H.; Morikawa, K. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 1442.
(97) Putnam, C. D.; Clancy, S. B.; Tsuruta, H.; Gonzalez, S.; Wetmur, J. G.; Tainer, J. A. *J. Mol. Biol.* **2001**, *311*, 297.
(98) Yamada, K.; Miyata, T.; Tsuchiya, D.; Oyama, T.; Fujiwara, Y.; Ohnishi, T.; Iwasakai, H.; Shinagawa, H.; Ariyoshi, M.; Mayanagi, K.; Morikawa, K. *Mol. Cell* **2002**, *10*, 671.
(99) Dunderdale, H. J.; Benson, F. E.; Parsons, C. A.; Sharples, G. J.; Lloyd, R. G.; West, S. C. *Nature* **1991**, *354*, 506.
(100) Iwasaki, H.; Takahagi, M.; Shiba, T.; Nakata, A.; Shinagawa, H. *EMBO J.* **1991**, *10*, 4381.
(101) Ariyoshi, M.; Vassylyev, D.; Iwasaki, H.; Nakamura, H.; Shinagawa, H.; Morikawa, K. *Cell* **1994**, *78*, 1063.
(102) Saito, A.; Iwaski, H.; Ariyoshi, M.; Morikawa, K.; Shinagawa, H. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7474.
(103) Bennett, R. J.; West, S. C. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 5635.
(104) Davies, A. A.; West, S. C. *Curr. Biol.* **1998**, *8*, 725.
(105) Van Gool, A. J.; Shah, R.; Mezard, C.; West, S. C. *EMBO J.* **1998**, *17*, 1838.
(106) Lloyd, R. G. *J. Bacteriol.* **1991**, *173*, 5414.
(107) Whitby, M. C.; Vincent, S. D.; Lloyd, R. G. *EMBO J.* **1994**, *13*, 5220.
(108) Lloyd, R. G. *J. Bacteriol.* **1991**, *173*, 1004.
(109) McGlynn, P.; Lloyd, R. G. *Cell* **2000**, *101*, 35.
(110) McGlynn, P.; Lloyd, R. G.; Marians, K. J. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8235.
(111) Singleton, M.; Scaife, S.; Wigley, D. *Cell* **2001**, *107*, 79.
(112) Sharples, G. J.; Ingleston, S. M.; Lloyd, R. G. *J. Bacteriol.* **1999**, *181*, 5543.
(113) McGlynn, P.; Lloyd, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8227.
(114) Briggs, G. S.; Mahdi, A. A.; Wen, Q.; Lloyd, R. G. *J. Biol. Chem.* **2005**, *280*, 13921.
(115) Sharples, G. J.; Ingleston, S. M.; Lloyd, R. G. *J. Bacteriol.* **1999**, *181*, 5543.
(116) Elborough, K.; West, S. C. *EMBO J.* **1990**, *9*, 2931.
(117) Hyde, H.; Davies, A. A.; Benson, F. E.; West, S. C. *J. Biol. Chem.* **1994**, *269*, 5202.
(118) Constantinou, A.; Davies, A. A.; West, S. C. *Cell* **2001**, *104*, 259.
(119) Constantinou, A.; Chen, X. B.; McGowan, C. H.; West, S. C. *EMBO J.* **2002**, *21*, 5577.
(120) Liu, Y.; Masson, J.-Y.; Shah, R.; O'Regan, P.; West, S. C. *Science* **2004**, *303*, 243.
(121) Komori, K.; Sakae, S.; Fujikane, R.; Morikawa, K.; Shinagawa, H.; Ishino, Y. *Nucleic Acids Res.* **2000**, *28*, 4544.
(122) Daiyasu, H.; Komori, K.; Sakae, S.; Ishino, Y.; Toh, H. *Nucleic Acids Res.* **2000**, *28*, 4540.
(123) Aravind, A.; Makarova, K. S.; Koonin, E. *Nucleic Acids Res.* **2000**, *28*, 3417.
(124) Lilley, D. M.; White, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 9351.
(125) Boddy, M. N.; Gailland, P. H.; McDonald, W. H.; Shanahan, P.; Yates, J. R.; Russell, P. *Cell* **2001**, *107*, 537.
(126) Chen, X.-B.; Melchionna, R.; Denis, C.-M.; Gailland, P.-H. L.; Blasina, A.; Van de Weyer, I.; Boddy, M. N.; Russell, P.; Vialard, J.; McGowan, C. H. *Mol. Cell* **2001**, *8*, 1117.
(127) Kaliraman, V.; Mullen, J. R.; Fricke, W., M.; Bastin-Shanower, S. A.; Brill, S. J. *Gene Dev.* **2001**, *15*, 2730.
(128) Doe, C. L.; Ahn, J. S.; Dixon, J.; Whitby, M. C. *J. Biol. Chem.* **2002**, *277*, 32753.
(129) Whitby, M. C.; Osman, F.; Dixon, J. *J. Biol. Chem.* **2003**, *278*, 6928.
(130) Ogrunc, M.; Sancar, A. *J. Biol. Chem.* **2003**, *278*, 21715.
(131) Ciccia, A.; Constantinou, A.; West, S. C. *J. Biol. Chem.* **2003**, *278*, 25172.
(132) Bastin-Shanower, S. A.; Fricke, W. M.; Mullen, J. R.; Brill, S. J. *Mol. Cell. Biol.* **2003**, *23*, 3487.
(133) Haber, J. E.; Heyer, W. D. *Cell* **2001**, *107*, 551.
(134) Prakash, L.; Prakash, S. *Genetics* **1977**, *86*, 33.
(135) Interthal, H.; Heyer, W. D. *Mol. Gen. Genet.* **2000**, *263*, 812.
(136) Xiao, W.; Chow, B. L.; Milo, C. N. *Mol. Gen. Genet.* **1998**, *257*, 614.
(137) Boddy, M. N.; Lopez-Girona, A.; Shanahan, P.; Interthal, H.; Heyer, W. D.; Russell, P. *Mol. Cell. Biol.* **2000**, *20*, 8758.
(138) Gaillard, P. H.; Noguchi, E.; Shanahan, P.; Russell, P. *Mol. Cell* **2003**, *12*, 749.

(139) Osman, F.; Dixon, J.; Doe, C. L.; Whitby, M. C. *Mol. Cell* **2003**, *12*, 761.
(140) Hollingsworth, N. M.; Brill, S. J. *Genes Dev.* **2004**, *18*, 117.
(141) Nishino, T.; Komori, K.; Ishino, Y.; Morikawa, K. *Structure* **2003**, *11*, 445.
(142) Nishino, T.; Komori, K.; Ishino, Y.; Morikawa, K. *Structure* **2005**, *13*, 1183.
(143) Newman, M.; Murray-Rust, J.; Lally, J.; Rudolf, J.; Fadden, A.; Knowles, P.; P.; White, M. F.; McDonald, N. Q. *EMBO J.* **2005**, *11*, 1.
(144) Nishino, T.; Komori, K.; Tsuchiya, D.; Ishino, Y.; Morikawa, K. *Structure* **2005**, *13*, 1.
(145) Sheller, J.; Schurer, A.; Rudolph, C.; Hettwer, S.; Kramer, W. *Genetics* **2000**, *155*, 1069.
(146) Schurer, K. A.; Rudolph, C.; Ulrich, H. D.; Kramer, W. *Genetics* **2004**, *166*, 1673.
(147) Prakash, R.; Krejci, L.; Van Komen, S.; Schurer, K. A.; Kramer, W.; Sung, P. *J. Biol. Chem.* **2005**, *280*, 7854.
(148) Amundsen, S. K.; Smith, G. R. *Cell* **2003**, *112*, 741.
(149) Horii, Z.; Clark, A. J. *J. Mol. Biol.* **1973**, *80*, 327.
(150) Kolodner, R.; Fishel, R. A.; Howard, M. *J. Bacteriol.* **1985**, *163*, 1060.
(151) Tseng, T. C.; Hung, J. L.; Wang, T. C. *Mutat. Res.* **1994**, *315*, 1.
(152) Nakayama, K.; Irino, N.; Nakayama, H. *Mol. Gen. Genet.* **1985**, *200*, 266.
(153) Morozov, V.; Mushegian, A. R.; Koonin, E. V.; Bork, P. *Trends Biochem. Sci.* **1997**, *22*, 417.
(154) Bernstein, D. A.; Keck, J. L. *Nucleic Acids Res.* **2003**, *31*, 2778.
(155) Bernstein, D. A.; Zittel, M. C.; Keck, J. L. *EMBO J.* **2003**, *22*, 4921.
(156) Karow, J. W.; Newman, R. H.; Freemont, P. S.; Hickson, I. D. *Curr. Biol.* **1999**, *9*, 597.
(157) Harmon, F. G.; Kowalcykowski, S. C. *J. Biol. Chem.* **2001**, *276*, 232.
(158) Kawasaki, K.; Maruyama, S.; Nakayama, M.; Matsumoto, K.; Shibata, T. *Nucleic Acids Res.* **2002**, *30*, 3682.
(159) Wu, L.; Hickson, I. D. *Mutat. Res.* **2002**, *509*, 35.
(160) Janscak, P.; Garcia, P. L.; Hamburger, F.; Makuta, Y.; Shiraishi, K.; Imai, Y.; Ikeda, H.; Bickle, T. A. *J. Mol. Biol.* **2003**, *330*, 29.
(161) Rodrigues, A. C.; Stock, D. *EMBO J.* **2002**, *21*, 418.
(162) Lovett, S.; Kolodner, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 2627.
(163) Yamagata, A.; Kakuta, Y.; Masui, R.; Fukuyama, K. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5908.
(164) Fujikane, R.; Komori, K.; Shinagawa, H.; Ishino, Y. *J. Biol. Chem.* **2005**, *280*, 12351.
(165) Fujikane, R.; Shinagaw, H.; Ishino, Y. *Genes Cells* **2006**, *11*, in press.
(166) Boyd, J. B.; Sakaguchi, K.; Harris, P. V. *Genetics* **1990**, *125*, 813.
(167) Oshige, M.; Aoyagi, N.; Harris, P. V.; Burtis, K.; Sakaguchi, K. *Mutat. Res.* **1999**, *433*, 183.
(168) Marini, F.; Wood, R. D. *J. Biol. Chem.* **2002**, *277*, 8716.
(169) Sharief, F. S.; Vojta, P. J.; Ropp, P. A.; Copeland, W. C. *Genomics* **1999**, *59*, 90.
(170) Seki, M.; Marini, F.; Wood, R. D. *Nucleic Acids Res.* **2004**, *31*, 6117.
(171) Guy, C. P.; Bolt, E. *Nucleic Acids Res.* **2005**, *33*, 3678.
(172) Lebel, M.; Spillare, E. A.; Harris, C. C.; Leder, P. *J. Biol. Chem.* **1999**, *274*, 37795.
(173) Huang, S.; Beresten, S.; Li, B.; Oshima, J.; Ellis, N. A.; Campisi, J. *Nucleic Acids Res.* **2000**, *28*, 2396.
(174) Rodriguez-Lopez, A. M.; Jackson, D. A.; Nehlin, J. O.; Iborra, F.; Warren, A. V.; Cox, L. S. *Mech. Ageing Dev.* **2003**, *124*, 167−174.
(175) Umezu, K.; Chi, N. W.; Kolodner, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 3875.
(176) Umezu, K.; Kolodner, R. D. *J. Biol. Chem.* **1994**, *269*, 30005.
(177) Hegde, S. P.; Qin, M. H.; Li, X. H.; Atkinson, M. A.; Clark, A. J.; Rajagopalan, M.; Madiraju, M. V. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14468.
(178) Beernink, H. T. H.; Morrical, S. W. *Trends Biochem. Sci.* **1999**, *24*, 385.
(179) Morimatsu, K.; Kowalczykowski, S. C. *Mol. Cell* **2003**, *11*, 1337.
(180) Lee, B. II.; Kim, K. H.; Park, S. J.; Eom, S. H.; Song, H. K.; Suh, S. W. *EMBO J.* **2004**, *23*, 2029.
(181) Leiros, I.; Timmins, J.; Hall, D. R.; McSweeney, S. *EMBO J.* **2005**, *24*, 906.
(182) Kreuzer, K. N. *Trends Biochem. Sci.* **2000**, *25*, 165.
(183) Kantake, N.; Madiraju, M. V.; Sugiyama, T.; Kowalczykowski, S. C. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15327.
(184) Kagawa, W.; Kurumizaka, H.; Ishitani, R.; Fukai, S.; Nureki, O.; Shibata, T.; Yokoyama, S. *Mol. Cell* **2002**, *10*, 359.
(185) Singleton, M. R.; Wentzell, L. M.; Liu, Y.; West, S. C.; Wigley, D. B. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 13492.
(186) Stasiak, A. K.; Larquet, E.; Stasiak, A.; Muller, S.; Engel, A.; VanDyck, E.; West, S. C.; Egelman, E. H. *Curr. Biol.* **2000**, *10*, 377.
(187) Cox, M. M.; Goodman, M. F.; Kreuzer, K. N.; Sherratt, D. J.; Sandler, S. J.; Marians, K. J. *Nature* **2000**, *404*, 37.
(188) Marians, K. *J. Philos. Trans. R. Soc. London B* **2004**, *359*, 71.
(189) Liu, J.; Xu, L.; Sandler S. J.; Marians, K. J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 3552.
(190) Jones, J. M.; Nakai, H. *J. Mol. Biol.* **1999**, *289*, 503.
(191) Tanaka, T.; Mizukoshi, T.; Taniyama, C.; Kohda, D.; Arai, K.; Masai, H. *J. Biol. Chem.* **2002**, *277*, 38062.
(192) Mizukoshi, T.; Tanaka, T.; Arai, K.; Kohda, D.; Masai, H. *J. Biol. Chem.* **2003**, *278*, 42234.
(193) Helleday, T. *Mutat. Res.* **2003**, *532*, 103.
(194) Hochegger, H.; Sonoda, E.; Takeda, S. *BioEssays* **2004**, *26*, 151.
(195) Branzei, D.; Foiani, M. *Curr. Opin. Cell Biol.* **2005**, *17*, 568.
(196) Stauffer, M.; Chazin, W. J. *J. Biol. Chem.* **2004**, *279*, 30915.

CR0404803