Multiple Interactions of the Intrinsically Disordered Region between the Helicase and Nuclease Domains of the Archaeal Hef Protein*§

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Background: Hef/FANCM participates in interstrand cross-link DNA repair.

Results: The predicted intrinsically disordered region (IDR) in Hef was experimentally verified. Proliferating cell nuclear antigen and a RecJ-like protein specifically bind to the IDR individually, but not simultaneously.

Conclusion: The IDR in Hef interacts with multiple proteins.

Significance: Hef may function in DNA repair by association of its IDR with multiple partners.

Hef is an archaeal protein that probably functions mainly in stalled replication fork repair. The presence of an unstructured region was predicted between the two distinct domains of the Hef protein. We analyzed the interdomain region of Thermococcus kodakarenensis Hef and demonstrated its disordered structure by CD, NMR, and high speed atomic force microscopy (AFM). To investigate the functions of this intrinsically disordered region (IDR), we screened for proteins interacting with the IDR of Hef by a yeast two-hybrid method, and 10 candidate proteins were obtained. We found that PCNA1 and a RecJ-like protein specifically bind to the IDR in vitro. These results suggested that the Hef protein interacts with several different proteins that work together in the pathways downstream from stalled replication fork repair by converting the IDR structure depending on the partner protein.

Hef is an archaeal protein that most probably functions mainly in stalled replication fork repair. It was originally discovered by screening for factors associating with the archaeal Holliday junction resolvase, Hjc, from a total cell extract of Pyrococcus furiosus, a hyperthermophilic archaeon (1). Identification of the gene corresponding to this activity revealed that the encoded protein consists of two distinct domains, with similarity to the DEAH helicase family and the XPF/Mus81 nuclease superfamily, respectively (1). Biochemical characterization of the purified protein confirmed the specific affinity for branched DNA structures, including the replication fork. The N-terminal domain possesses ATPase and helicase activities that were dramatically stimulated by fork-structured DNAs, and the C-terminal domain has an endonuclease activity that specifically cleaves nicked, flapped, and fork-structured DNAs (1, 2). Based on these properties, this protein was designated as Hef (helicase-associated endonuclease for fork-structured DNA), and it was proposed that Hef may be a prototypical enzyme for the eukaryotic XPF/Rad1/Mus81 nuclease family (2, 3).

A genetic manipulation system was developed for Thermococcus kodakarenensis (4), and phenotype analyses of the hef mutant strain confirmed that Hef is involved in multiple repair processes, and its especially high sensitivity to mitomycin C suggested that Hef performs a critical function in DNA interstrand cross-link repair (5). The helicase and nuclease activities of the purified T. kodakarenensis Hef protein (TkoHef) for fork-structured DNA was also confirmed in vitro (5). These genetic and biochemical properties of TkoHef suggested that this protein actually works at stalled replication forks in T. kodakarenensis by the coordination of its helicase and endonuclease activities, as predicted.

Hef is well conserved in Euryarchaeota. Genetic and cytological analyses of the hef gene in Halofexus volcanii also revealed that Hef is involved in stalled replication fork repair (6, 7). On the other hand, the crenarchaeal organisms have a protein comprising only the endonuclease domain, and its nuclease activity for branched DNA is completely proliferating cell nuclear antigen (PCNA)2-dependent (8, 9).

Research on stalled replication fork repair in Eukarya is now actively progressing (10–13). Defects in this repair system lead to diseases with symptoms including developmental abnormalities and a predisposition to cancer. A well known hereditary disease related to this repair system is Fanconi anemia (FA).
Intrinsically Disordered Region of Hef/FANCM

TABLE 1

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>Hef-F</td>
<td>5′-dGGGCGGCCGCTTTAGGGAATCTTCTTCAGCTTCACG-3′</td>
</tr>
<tr>
<td>Hef-R</td>
<td>5′-dGGGCGGCCGCTTTAGGGAATCTTCTTCAGCTTCACG-3′</td>
</tr>
<tr>
<td>del-F</td>
<td>5′-dGGGCGGCCGCTTTAGGGAATCTTCTTCAGCTTCACG-3′</td>
</tr>
<tr>
<td>del-R</td>
<td>5′-dGGGCGGCCGCTTTAGGGAATCTTCTTCAGCTTCACG-3′</td>
</tr>
<tr>
<td>HefT695D-F</td>
<td>5′-dGGGCGGCCGCTTTAGGGAATCTTCTTCAGCTTCACG-3′</td>
</tr>
<tr>
<td>HefT695D-R</td>
<td>5′-dGGGCGGCCGCTTTAGGGAATCTTCTTCAGCTTCACG-3′</td>
</tr>
<tr>
<td>HefT600stop-F</td>
<td>5′-dGGGCGGCCGCTTTAGGGAATCTTCTTCAGCTTCACG-3′</td>
</tr>
<tr>
<td>HefT600stop-R</td>
<td>5′-dGGGCGGCCGCTTTAGGGAATCTTCTTCAGCTTCACG-3′</td>
</tr>
<tr>
<td>ID-F</td>
<td>5′-dGGGCGGCCGCTTTAGGGAATCTTCTTCAGCTTCACG-3′</td>
</tr>
<tr>
<td>ID-R</td>
<td>5′-dGGGCGGCCGCTTTAGGGAATCTTCTTCAGCTTCACG-3′</td>
</tr>
<tr>
<td>TK0155-F</td>
<td>5′-dGGGCGGCCGCTTTAGGGAATCTTCTTCAGCTTCACG-3′</td>
</tr>
<tr>
<td>TK0155-R</td>
<td>5′-dGGGCGGCCGCTTTAGGGAATCTTCTTCAGCTTCACG-3′</td>
</tr>
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Currently, 16 genes, designated as FANCA to FANCP, have been identified in the FA-related DNA repair pathway (14–18). Among these gene products, the FANCM protein works as a molecular scaffold with FAAP24 and MHPF proteins to recruit the FA core complex, containing FANCA, -G, -C, -E, -F, -B, -L, and FAAP100, for the monoubiquitination of FANCD2 and FANCI. These modifications activate the FANCD2-A complex to recruit other related proteins, including BRCA1, BRCA2, FANCN, and FANCJ, to the damaged site. Reports in 2005 that human and chicken FANCMs are orthologs of the archaeal Hef (19, 20) stimulated progress of the research on the molecular mechanisms of FA repair pathways.

Comparisons of the structures of the archaeal Hef and eukaryotic FANCM proteins led us to focus on the function of the region between the N-terminal helicase and C-terminal nuclease, because the region seemed to be intrinsically disordered. Intrinsically disordered proteins (IDPs), which lack a stable three-dimensional structure, are now attracting attention in both protein chemistry and molecular biology fields, because the structural plasticity of IDPs confers functional advantages (21–24). The structural plasticity enables the proteins to bind structurally distinct target molecules by structural conversion. The IDPs are also able to provide a larger surface as an interface, to interact with other molecules, and to escape steric hindrances. These properties are advantageous for interactions with numerous partners with high specificity and low affinity. Therefore, the IDPs function in many life processes by interacting with several partner molecules in a time-dependent manner.

In this study, we performed structural and functional analyses of the intrinsically disordered region (IDR) in T. kodakarenensis Hef. The predicted IDR was experimentally confirmed to be unstructured. The interaction of IDR with several proteins, including PCNA and a RecJ-like protein, suggest that Hef uses its IDR for sequential bindings of several proteins to process the repair pathway as a scaffold at the stalled fork.

**EXPERIMENTAL PROCEDURES**

**Prediction of IDR in the Hef Family Proteins**—To predict the IDR in TkoHef and human FANCM, the Hef/FANCM proteins were subjected to a GTOP database (25) search and a DICHOT prediction analysis (26), which divide proteins into structural domains and IDR with high accuracy.

**Production and Purification of TkoHef**—Cloning of the hef gene and preparation of the TkoHef protein were described in detail in our previous report (5). For the preparation of the His-tagged IDR (His-ID), the gene fragment encoding Met K900–Lys593 of TkoHef (Hef-ID) was amplified by PCR from T. koda-karenensis DNA, using the primer set ID-F/ID-R including Ndel and NotI recognition sequences at their 5′-regions (shown in Table 1) for insertion into the Ndel-NotI sites of a modified pET-28a (+) vector (Novagen). The modification of pET-28a (+) was the conversion of the thrombin recognition sequence to the TEV protease recognition sequence. The resultant plasmid was designated as pET28a(TEV)-ID. *Escherichia coli* BL21-CodonPlus (DE3)-RIL cells (Agilent) harboring pET28a(TEV)-ID were grown at 37 °C in 1 liter of LB medium, containing 50 μg/ml of kanamycin and 34 μg/ml of chloramphenicol. When the *E. coli* culture reached an A600 of 0.3, expression of the gene encoding His-ID was induced by adding isopropyl β-D-thiogalactopyranoside to 1 mM. After cultivation at 25 °C for 16 h, the cells were harvested by centrifugation, and the cell lysate was prepared by sonication in 40 ml of buffer A (50 mM sodium phosphate, pH 7.8, 0.5 mM NaCl, and 1 mM PMSF). After centrifugation for 20 min at 23,700 × g, the supernatant was applied to a His-affinity column (HisTrap HP, GE Healthcare), which was eluted with an imidazole gradient from 10 mM to 0.5 M. The fractions containing His-ID were dialyzed against buffer B (50 mM Tris-HCl, pH 8.0, 0.5 mM DTT, 0.1 mM EDTA) including 0.1 M NaCl, and then subjected to cation exchange (HiTrap SP HP, GE Healthcare) chromatography and gel filtration (Superdex 200, GE Healthcare) chromatography.

**Preparation of Mutant TkoHefs**—For the construction of the IDR deletion mutant (TkoHefΔID), four primers were designed to fuse the two fragments via PCR. These primer sequences are listed in Table 1. The DNAs encoding the N-terminal (1,476 nucleotides) and C-terminal (669 nucleotides) fragments were amplified individually, using primer sets Hef-F/del-R and del-F/Hef-R, respectively. A second PCR was performed to combine the N-terminal and C-terminal fragments, using a mixture of the amplified fragments. A third PCR was performed using the amplified fragment mixture as the DNA templates, with primer set Hef-F/del-R and del-F/Hef-R, respectively. The resultant plasmid was designated as pET21a-Hef-del. Amino acid substitutions for TkoHef were introduced into the hef gene by PCR-mediated mutagenesis (QuickChange site-directed mutagenesis kit; Stratagene), by sequentially using primers HefT695D-F/HefT695D-R and
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HefT800stop-F/HefT800stop-R (Table 1), to make a TkoHef mutant (TkoHef-m) that cannot form a stable homodimer. The preparation of TkoHefΔID and TkoHef-m proteins was performed according to the same procedure used for the TkoHef protein. The purified fractions were concentrated and stored at 4 °C.

Preparation of TK0155 (HAN)—The genes for TK0155 were amplified by PCR from *T. kodakarensis* DNA, using the primer set including NdeI and NotI recognition sequences at their 5’-regions (shown in Table 1) for insertion into the NdeI-NotI sites of pET-21a (+). The resultant plasmid was introduced into *E. coli* BL21-CodonPlus (DE3)–RIL cells and grown at 37 °C in 1 liter of LB medium, containing 50 μg/ml of ampicillin and 34 μg/ml of chloramphenicol. When the *E. coli* culture reached an *A*_600 of 0.3, expression of the genes were induced by adding isopropyl β-D-thiogalactopyranoside to 1 mM. After cultivation at 25 °C for 16 h, the cells were harvested, and the cell lysate was prepared by sonication in 40 ml of buffer B including 1 M NaCl. The soluble cell extracts were heated at 80 °C for 20 min. The heat-resistant fraction was subjected to treatment with 0.15% polyethyleneimine. The proteins in the supernatants were precipitated by 80% saturated ammonium sulfate. The precipitate was resuspended in buffer B containing 0.5 M ammonium sulfate, and subjected to chromatography on a 5-ml HiTrap Phenyl HP column, which was developed with a linear gradient of 0.5–0 M ammonium sulfate. The eluted protein fractions were dialyzed against buffer B, and then loaded onto a 5-ml HiTrap Q HP column (GE Healthcare), which was developed with a linear gradient of 0–4 M NaCl.

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**Nuclease Assay**—A substrate single-stranded DNA (5′-CGA-ACGCGCCCTGGAAATCTGAGCAATGGACTT) was labeled at the 3′ terminus with FITC (Hokkaido System Science Co., Ltd.). TK0155 (HAN) (50 nM) was incubated with 100 nM single-stranded DNA in 20 μl of reaction buffer (25 mM Tris–HCl, pH 8.0, 2 mM MgCl₂, 0.1 mg/ml of BSA, and 1 mM DTT) at the indicated temperature for 10 min. Reactions were terminated with 5 μl of stop solution (15% Ficoll, 50 mM EDTA, 1% SDS, and 0.1% bromphenol blue). The products were analyzed by 15% PAGE with 0.5× TBE. FITC-labeled DNA was visualized by a Typhoon Trio+ image analyzer (GE Healthcare).

**Limited Proteolysis**—TkoHef and TkoHefΔID (0.3 mg/ml of protein) were incubated with trypsin (Sigma) at a 1:500 enzyme:substrate (w/w ratio) in 50 mM Tris–HCl, pH 8.0, 50 mM NaCl at 37 °C for 1, 3, 10, and 20 min. Reactions were stopped by the addition of PMSF at a final concentration of 10 mM. Aliquots were subjected to 12.5% SDS-PAGE followed by staining with Coomassie Brilliant Blue.

**Circular Dichroism Measurements**—The CD spectra were collected on a J-820 spectropolarimeter (JASCO, Tokyo, Japan) at room temperature, between 190 and 250 nm. The time constant, scan speed, bandwidth/resolution, and sensitivity of the spectropolarimeter were set at 1 s, 50 nm/min, 1 nm, and 100 mdeg, respectively. A solution of 20 μM protein in 50 mM sodium phosphate buffer, pH 6.8, was measured at 190–250 nm. The protein solutions in 1, 4, and 8 M urea were measured at 210–250 nm. The presence of urea prevented the measurement below 210 nm due to the high absorbance of the solution. The quantity of α-helix was evaluated from the signal at 222 nm using Equation 1 (28).

![Equation 1](http://www.jbc.org/)

**NMR Analysis**—NMR experiments were performed on a Bruker AvanceIII 600 MHz NMR spectrometer equipped with a cryogenic probe and pulsed-field gradients. To assess whether the IDR of the TkoHef protein (Hef-ID) exhibits properties of IDPs, we employed a “chimeric membrane protein”-based NMR method. In brief, a *Pyrococcus horikoshii* PH0471 membrane protein was engineered, and its inter-domain linker region (residues 72–83) was replaced with Hef-ID. The chimeric membrane protein PH0471-(Hef-ID) was expressed in *E. coli*, purified, and solubilized in dodecyl maltoside micelles. For the NMR experiments, ~0.8 mg of [15N]PH0471-(Hef-ID) was dissolved in 0.30 ml of H₂O/O₂D₂O (9:1), containing 25 mM sodium phosphate buffer, pH 5.0, 100 mM NaCl, and 0.1% (w/v) dodecyl maltoside micelles. HSQC spectra (29) modified with gradient sensitivity enhancement (30) were acquired with 32 transients and 256 increments at 303 K. The control spectra (PH0471 wild type (WT) and NheID domain only) were both recorded with a Bruker DRX 500 MHz spectrometer at 298 K. All two-dimensional spectra were processed with nmrPipe (31) and analyzed with the program nmrDraw (31).

**High Speed Atomic Force Microscopy (AFM) Imaging**—A laboratory-built high speed AFM was used for observations of the TkoHef proteins. The instrument was previously described in detail (32, 33). AFM images were acquired in the tapping mode. Cantilevers were specially designed for high speed imaging, and provided by Olympus. The resonant frequency was 0.8–1.2 MHz in water, and the spring constant was 0.1–0.2 newton m⁻¹. The quality factor of the cantilevers in water was about 2. An amorphous carbon tip, additionally grown on the tip of a cantilever by electron beam deposition, was used as the AFM probe. For high speed AFM observations, a droplet (2 μl) of the solution containing ~1 nM TkoHef (WT or mutant proteins) was deposited on a freshly cleaved mica surface. After incubation for 3 min, the samples that were not attached to the mica surface were removed by rinsing with buffer solution, and the AFM observation was then performed with the same buffer solution. The buffer solutions used for observations of the WT TkoHef and the other mutants were 300 mM NaCl, 10 mM HEPES-NaOH, pH 7.4, and 30 mM NaCl, 10 mM HEPES-NaOH, pH 7.4, respectively. The high salt condition was important to keep the dimerized form of the WT TkoHef on the mica surface. The heights and the end-to-end length analyses for determining the molecular features were performed as previously described (34).

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*Immunoprecipitation Experiment*—A 20-μl portion of rProtein A Sepharose Fast Flow (GE Healthcare Biosciences) was washed three times with PBS-T (10 mM sodium phosphate, pH 7.5, 150 mM NaCl, 0.1% Tween 20), mixed with 10 μl of anti-TkoPCNA1 (35) or anti-TkoHef antiserum (5), and incubated at room temperature for 1 h on a rotary shaker. Each mixture was washed twice with PBS-T, and then twice with 0.2 M triethanolamine, pH 8.0. The antibody was cross-linked to Protein A with dimethyl suberimidate/2/HC1 (Pierce), according to the manufacturer’s protocol. For the negative control, preimmune antiserum was used. *T. kodakarensis* cells (2.5 × 10^13 cells) were disrupted by sonication in 15 ml of lysis buffer (50 mM Tris-HCl, pH 8.0, 50 mM NaCl, 0.5 mM dithiothreitol, 0.1 mM EDTA, 0.1% Triton X-100, and 10% glycerol) containing proteinase inhibitor (Complete™, Roche Applied Science), and the extract was obtained by centrifugation. After equilibration of the antibody-conjugated rProtein A Sepharose with lysis buffer, a 400-μl aliquot of *T. kodakarensis* cell extract was added, and the mixture was incubated for 30 min on a rotary shaker. The precipitates were washed three times with lysis buffer, and the immunoprecipitated proteins were eluted with 40 μl of gel loading solution (50 mM Tris-HCl, pH 6.8, 1% glycerol, 5% β-mercaptoethanol, 0.2% bromphenol blue, 2% SDS). The protein samples were separated by SDS-12% PAGE, electroblotted onto a PVDF membrane, and subjected to Western blot analysis. Proteins were visualized by an enhanced chemiluminescence system (Millipore) and LAS-3000 mini-image analyzer (Fujifilm).

*Surface Plasmon Resonance (SPR) Analysis*—A Biacore I (GE Healthcare) system was used to study the physical interactions between the proteins. All measurements were conducted at 25 °C. Purified recombinant TkoPCNA1 (35), TK0155 (HAN), and His-ID were bound to CM5 sensor chips individually, according to the manufacturer’s recommendations. The concentrations of the analyte proteins and the compositions of the running buffer are described in the respective sections. At the end of each cycle, the bound proteins were removed by washing with 2 mM sodium chloride. The apparent equilibrium constants (K_a) were determined from the association and dissociation curves of the sensorgrams, using the BIAevaluation program (GE Healthcare).

*Two-hybrid Assay*—A yeast two-hybrid (Y2H) detection system (Matchmaker™ Gold Yeast Two-hybrid System, Matchmaker GAL4 Two-Hybrid System 3, Clontech) was used to screen for Hef-ID-interacting proteins. The plasmid pGBK7T, encoding the GAL4 DNA binding region, and the plasmid pGAD7, encoding the activation domains, were used to prepare plasmids containing the gene encoding Met490-Lys593 of TkoHef and various genomic DNA fragments, respectively. The gene fragment encoding Met490-Lys593 was amplified from *T. kodakarensis* DNA using the two primers including NdeI and NotI recognition sequences at their 5′-regions (shown in Table 1), for insertion into the NdeI-NotI sites of pGBK7T. For the preparation of a prey library, *T. kodakarensis* DNA was fragmented by sonication (Microson Ultrasonic Cell Disruptor, Misonix) and 0.5–1.5-kb DNA fragments were fractionated and eluted from an agarose gel. These DNA fragments were subjected to blunting with T4 DNA polymerase and *E. coli* Klenow fragment, ligated with the EcoRI-NotI-BamHI adaptor, and inserted into the EcoRI site of pGADT7, after cleavage by EcoRI. The ligation solution was mixed with *E. coli* MegaX DH10B, and the cells were transformed by electroporation. The size of the prepared library was 3.1 × 10^8. Co-transformations of the yeast Y2H Gold cells with pGBK7T-Hef ID and the pGAD7 library were performed according to the manufacturer’s protocol (Clontech Matchmaker manual). Cells were cultured on SD plates containing Aureobasidin A without Leu and Trp at 30 °C for 3 days for selection. The cell suspensions from the obtained colonies were spotted onto SD plates containing Aureobasidin A with or without His. The plates were incubated at 30 °C, and the growth of the yeast cells was monitored every 24 h for 4 days. The cell growth on the plates indicated the interactions of the two proteins produced from the two plasmids used for the co-transformation.

**RESULTS**

*Prediction of the IDRs in the Hef/FANCM Proteins*—The sequences of the helicase and nuclease domains are conserved in Archaea and Eukarya through evolution, but the central regions are highly divergent in the Hef/FANCM proteins. The amino acid sequence analysis of the archaeal Hefs searched in the GTOP database clearly predicted that the inter-domain region (residues 493 to 597, 105 amino acids) is entirely disordered (Fig. 1A). The location of the disordered region between the two distinct domains was also predicted for the eukaryotic FANCM proteins by DICHOT. Their unstructured regions are quite large (about 1,243 amino acids long, including residues 599 to 1,841 for human FANCM) because the eukaryotic FANCMs have long peptide chains (2,048 amino acids) (Fig. 1B). These long disordered structures in the Hef/FANCM proteins should have physiological meanings. The sequences of the archaeal Hef proteins in the public database were subjected to the domain analysis, which revealed that the structural composition, helicase-ID-nuclease, is conserved in Hef proteins found in Euryarchaeota (Fig. 1C). It is evolutionally interesting that the other phyla of Archaea have only the nuclease domain (Crenarchaeota) or the helicase domain (Nanoarchaeota), or separated helicase and nuclease domains (Thaumarchaeota), and the IDR is found in the C-terminal regions of the helicase domain (Fig. 1C).

*Protein Purifications*—To characterize Hef-ID, the recombinant IDR protein with the N-terminal His tag, His-ID, was successfully overproduced. The protein was purified to near homogeneity by the three sequential His tag, His-ID, was successfully overproduced. The protein was purified to near homogeneity by the three sequential chromatography steps described in Fig. 2A. From a 1-liter culture (3.4 g cells), 1.49 mg of homogeneous protein was obtained. In addition, we purified two Hef mutant proteins that are deficient in dimer formation, TkoHef-m (based on knowledge from our structural studies of *P. furiosus* Hef (36)) and TkoHefΔID, with a deletion of the IDR(493–588), as well as the WT Hef protein. The purified proteins are shown in Fig. 2, B and C.

*Limited Proteolysis*—Accessibility and flexibility of the substrate affect on the cleavage efficiency of the proteases, and, disordered regions are generally easy targets for proteolytic attack. Therefore, using purified TkoHef and TkoHefΔID as described above, trypsin reaction was performed with a limited
As shown in Fig. 3A, the WT TkoHef was cleaved by trypsin to two major parts with increasing reaction time. On the other hand, TkoHef/H9004ID showed a highly resistant feature to the trypsin reaction (Fig. 3B). The cleavage products of TkoHef correspond to the sizes of the helicase and nuclease domains, respectively (Fig. 3C). Several bands were observed around each cleaved product (Fig. 3A), suggesting that TkoHef was cleaved at several different sites in the ID region, which contains many Lys and Arg residues. These results indicate that TkoHef has trypsin-susceptible sites at the predicted ID region.

Structural Analyses of the Hef-ID—The CD spectrum of the purified His-ID protein was recorded in the wavelength range of 190–250 nm (Fig. 4A). The spectrum in a phosphate buffer solution was characterized by a shoulder at 222 nm and an intense negative minimum at 202 nm. These features reveal that His-ID is mostly disordered, but contains some helical structure (7%), which was calculated as described earlier (28), followed by subtraction of the value in the case of the spectrum with 8 M urea. For further structural analysis of the disordered property of Hef-ID by NMR, we used our chimeric membrane protein-based systematic NMR assessment system for IDP. In this system, the target protein of interest (Hef-ID in this study) was expressed as a fusion with an archaeal membrane protein, PH0471, which provides a well known HSQC spectrum, as condition. As shown in Fig. 3A, the WT TkoHef was cleaved by trypsin to two major parts with increasing reaction time. On the other hand, TkoHefΔID showed a highly resistant feature to the trypsin reaction (Fig. 3B). The cleavage products of TkoHef correspond to the sizes of the helicase and nuclease domains, respectively (Fig. 3C). Several bands were observed around each cleaved product (Fig. 3A), suggesting that TkoHef was cleaved at several different sites in the ID region, which contains many Lys and Arg residues. These results indicate that TkoHef has trypsin-susceptible sites at the predicted ID region.

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**FIGURE 1. IDR in the TkoHef and FANCM proteins.** The classifications of TkoHef in the GTOP database (ID code BAD85210.1) (A) and human FANCM by DICHOT prediction (ID code FANCM_HUMAN) (B) are presented. The structural regions with similarity to known three-dimensional structures are shown with PDB codes under the bars, and unstructured regions are colored black. The numbers above the black bars indicate the amino acid residues of unstructured regions. The domains are shown by the arrows. C, domain analyses of archaeal Hef homologs from each subdomain are shown. The helicase and nuclease domains are shown in gray and white, respectively. IDRs are black. The classification of unstructured regions is according to the DISOPRED lines in the GTOP database. GenBank accession numbers used in GTOP are as follows. T. kodakarensis, BAD85210.1; P. furiosus, AAL82139.1; P. abyssi, CAB49203.1; Methanoscina maezi, AAM31083.1; Archaeoglobus fulgidus, AAB89786.1; Methanococcus jannaschii, AAB95187.1; Halobacterium salinarum, CAP14762.1; Methanothermobacter thermautotrophicus, AAB85892.1; Thermoplasma acidophilum, Ta1501.1; Sulfolobus solfataricus, eIF4A; Candidatus K. cryptofilum, ACB00930.1; Nanoarchaeum equitans, AAR39218.1; Cenarchaeum symbiosum, ABK77806.1, ABK76661.1; Nitrosopumilus maritimus, ABX128401.1, ABX123333.1.

**FIGURE 2. Purification of TkoHef and related proteins in this study.** A, purified His-ID (1 μg) was fractionated by 12.5% SDS-PAGE. B, TkoHef and TkoHefΔID (1 μg each) were fractionated by 7% SDS-PAGE on the same gel. C, TkoHef-m (1 μg) was fractionated by 10% SDS-PAGE. The proteins were stained by Coomassie Brilliant Blue. Protein markers (lane 1) were obtained from New England Biolabs Inc.
In the $^1$H-$^{15}$N HSQC spectrum of the PH0471-Hef-ID fusion protein, 73 signals of the 179 observed signals (Fig. 4B) were exactly the same as those from the PH0417 protein by itself (Fig. 4D), mostly corresponding to the signals from the isolated C-terminal NfeD domain (Fig. 4C). This result suggested that the Hef-ID part was highly flexible in solution. In addition, 89 additionally observed signals originating from NH groups of the Hef-ID (96%) showed the limited chemical shift dispersion within 8.6 and 7.8 ppm (Fig. 4B). These results clearly supported our prediction that the IDR of TkoHef in the fusion protein is predominantly unstructured and flexible.

The IDR Contributes to the Flexible Motion of the Two Distinct Domains of TkoHef—AFM visualizes individual protein molecules directly as a frame shot of time-averaged features. In combination with a high speed recording technique, images of the structural changes of a single molecule can be captured. This technique was used to observe the TkoHef protein to determine whether the IDR is actually disordered in the TkoHef protein. In the case of WT TkoHef, two large globules were connected to a slightly smaller globule by thin flexible linkers, and the globules, especially the smaller one, moved rapidly, as shown in Fig. 5A (see supplemental Movie AFM-S1). The images from TkoHef-m revealed that only one large glob-
ule was connected to a small globule by a thin flexible linker, and the small globule moved rapidly, as shown in Fig. 5B (see supplemental Movie AFM-S2). These results demonstrated that the TkoHef protein forms a dimer through the small globule. According to the crystal structure of *P. furiosus* Hef (36, 37), the helicase domain is larger than the nuclease domain. Thus, the large and small globules represent the helicase and nuclease domains of TkoHef, respectively. These assignments are also supported by our previous report showing that *P. furiosus* Hef formed a dimer at the nuclease domain (2). Note that the characteristic “c”-like shape of the crystal structure of the helicase domain (37) was sometimes observed in the large globule (for example, at 0.40 and 4.22 s in Fig. 5B), providing further evidence that the large globule is the helicase domain. On the other hand, in the case of TkoHefΔID, the large and small globules were connected to each other, and no flexible linker was observed, as shown in Fig. 5C (see supplemental Movie AFM-S3). The heights of the large and small globules were 3.6 ± 0.5 (n = 2,717) and 3.0 ± 0.6 nm (n = 2,717) for TkoHef-m (Fig. 6, D and E), and 3.6 ± 0.4 (n = 1,317) and 2.9 ± 0.4 nm (n = 1,317) for TkoHefΔID (Fig. 6, G and H), respectively, which are very similar to each other. The only visible difference between the AFM images of the two constructs was the existence of the flexible linker. Thus, it can be concluded that the flexible linker seen in TkoHef-m is the IDR of TkoHef. On the other hand, the height of the large globule was 4.4 ± 0.6 nm (n = 4,540) for TkoHef (Fig. 6A), which is higher than those of TkoHef-m and TkoHefΔID. This height variation may be caused by the different observation conditions. The observations of TkoHef were performed under conditions with high salt buffer, which often weakens the interactions between proteins and the mica surface, and thus slightly increases the height of proteins due to enhanced structural flexibility. The height of the small globule of TkoHef is 3.6 ± 0.6 nm (n = 2,270, Fig. 6B), which is also higher than those of TkoHef-m and TkoHefΔID. This may be caused by the combined effects of the different observation buffers and the dimerization of this domain. Furthermore, the mean end-to-end lengths of the flexible linker for the WT and TkoHef-m proteins were 14 ± 5 (n = 4,540) and 14 ± 6 nm (n = 2,717), respectively (Fig. 6, C and F). Because these values are similar to each other, the flexible linker detected in the WT must also be the IDR region. The maximum end-to-end length, which may be very close to the contour length, of the IDR region for the WT and monomer mutant were 32 (among 4,540 frames) and 35 nm (among 2,717 frames), respectively (Fig. 6, C and F). Assuming that the average distance between the nearest neighbor amino acids is 0.34 nm, and that the IDR region of TkoHef is composed of 104 amino acids, these values seem to be quite reasonable (0.34 nm × 104 = 35.4 nm). The parameters obtained here may not reach the most probable values, because only three of the typical molecules for each construct using 1,317–2,717 frames were analyzed here. However, we
believe that these parameters are convincing for our conclusions because they represent well the molecular features of each construct. Taken together, these AFM observations directly demonstrated the molecular features of the three TkoHef proteins, and suggested that the predicted IDR actually exists between the helicase and nuclease domains. The IDR moved rapidly and lacked a distinct structure, which may facilitate structural conversions for the different functions of TkoHef in various cellular phenomena.

Interaction between TkoPCNA1 and TkoHef—We predicted that the IDR of TkoHef functions in interactions with various partner molecules with different binding modes, by coupled binding and folding, and thus we searched for the IDR-binding proteins. PCNA is well known as a scaffold molecule that interacts with numerous proteins involved in DNA replication and repair. Therefore, an immunoprecipitation experiment was performed using a T. kodakarensis cell extract, the antisera against TkoPCNA1, one of the two PCNAs in this organism, and the antisera against TkoHef. As shown in Fig. 7A, the TkoPCNA1 band was detected in the fraction precipitated with the anti-TkoHef antibody. Conversely, TkoHef was co-precipitated with TkoPCNA1 by the anti-TkoPCNA1 antibody (Fig. 7B). A faint band of TkoPCNA1 was detected in the mock lane (precipitated with the preimmune antiserum). However, quantifications of each band in this experiment (repeated five times) showed significant differences of the band intensities between the co-precipitation lanes and mock lanes. Although it would be possible that these two proteins were co-precipitated through DNA, but not by direct interaction, these results supported that a complex containing both TkoPCNA1 and TkoHef exists, at least in T. kodakarensis cells. The cell extract was prepared by sonication with a condition that most of the DNA strands were digested to very short fragments, and therefore,
we thought the co-precipitation of TkoPCNA1 and TkoHef probably resulted from their direct interaction. To confirm the direct interaction, we performed SPR experiments with the purified TkoHef and TkoPCNA1 proteins. The TkoPCNA1 protein was covalently immobilized on the Biacore CM5 sensor chip, and different concentrations of the TkoHef protein were subsequently injected. The SPR sensorgram clearly showed the physical interaction between the TkoHef and TkoPCNA1 proteins (Fig. 7C). The calculated $K_D$ value for the interaction was $5.3 \times 10^{-7} \text{M}$, which is comparable with the values for other PCNA-binding proteins in *P. furius* (1.1 $\times 10^{-7} \text{M}$ for DNA ligase, 9.9 $\times 10^{-8} \text{M}$ for DNA polymerase B1, and 2.2 $\times 10^{-7} \text{M}$ for uracil DNA glycosylase), as determined by SPR analyses in our laboratory (38–40).

### Table 2

<table>
<thead>
<tr>
<th>Locus tag</th>
<th>Annotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TK0155</td>
<td>RecJ-like exonuclease</td>
</tr>
<tr>
<td>TK0356</td>
<td>Hypothetical membrane protein</td>
</tr>
<tr>
<td>TK0467</td>
<td>Hypothetical protein TK0467</td>
</tr>
<tr>
<td>TK0522</td>
<td>Carbohydrate esterase family 1 protein</td>
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<tr>
<td>TK1781</td>
<td>Diaminopimelate aminotransferase</td>
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<tr>
<td>TK1997</td>
<td>Hydrogenase maturation protein HypF</td>
</tr>
<tr>
<td>TK2021</td>
<td>ParA/MinD family ATPase</td>
</tr>
<tr>
<td>TK2303</td>
<td>Chaperonin $\beta$ subunit</td>
</tr>
</tbody>
</table>

**Intrinsically Disordered Region of Hef/FANCM**

The internal region of a peptide chain (38, 40). We subjected ible areas, such as looped-out regions, when present in the IDR, because PCNA-binding sites also exist in structurally flex-

dicted that the PCNA-binding site of TkoHef is located in the internal region of a peptide chain (38, 40). We subjected TkoHef to the same SPR analysis. As shown in Fig. 8A, no response was observed in the sensorgram for TkoHefID. Furthermore, the purified His-ID protein showed a clearly positive sensorgram, although the calculated $K_D$ value, 9.1 $\times 10^{-6} \text{M}$, was 1 order of magnitude higher than that of the WT TkoHef (Fig. 8B). All of the data supported the proposal that TkoHef uses its IDR to bind to TkoPCNA1.

**Exhaustive Search for Proteins Interacting with the IDR of TkoHef**—One of the important functions of IDR is to interact with plural partners by converting its conformation. Therefore, we tried an exhaustive screening of the candidate proteins that interact with TkoHef-ID by the Y2H experiment. The gene encoding TkoHef-ID was cloned into the bait plasmid pGBK7T7, as a fusion with the gene encoding the GAL4 DNA binding domain. A genomic DNA library was constructed using *T. kodakarensis* DNA and the prey plasmid pGAD7T7, as fusions with the genes encoding the GAL4 activation domain, as described under “Experimental Procedures.” Fourteen clones grew on the selection plates. The prey plasmids were then prepared from these positive clones, and the nucleotide sequences of the inserted fragments were determined. A public database search for the genes providing positive signals revealed each NCBI locus tag (TK number), and 10 different genes were identified among the 14 clones. In the case of four genes (TK0356, TK0467, TK1577, and TK2303) among the 14 clones, two clones each with different regions were included. The positive clones were cultivated individually and a portion for each liquid culture was spotted on the selection plate again (Fig. 9). Four of the 14 clones grew on the selection plates with the empty bait plasmid, and these self-activating clones, one of TK0356, TK0669, and both of TK1577, were excluded from the candidates. Most of the gene products are annotated as hypothetical proteins, as shown in Table 2, and the functions of these proteins should be analyzed individually. One interesting candidate, TK0155, was a bacterial RecJ-like protein. RecJ is a 5’-3’ exonuclease involved in several DNA repair pathways. Therefore, TK0155 may be a participant in the repair process, after Hef forms a repairosome complex at the stalled replication fork. The gene encoding TK0155 was cloned, and the gene product was produced as a full-length protein in *E. coli* cells (Fig. 10A). The purified TK0155 protein was confirmed to have the exonuclease activity, which is optimal at higher temperatures (Fig. 10B). An SPR analysis was performed, using the His-ID-immobilized sensor chip and the purified TK0155 protein, and binding of the two proteins was quantitatively confirmed, with an apparent $K_D$ value of 3.6 $\times 10^{-6} \text{M}$ (Fig. 11A). Based on these
Intrinsically Disordered Region of Hef/FANCM

Although the sequences are divergent among species, their disordered region (104 amino acids long) of Hef-ID will be flexibly folded depending on its binding partners. The adoption of different forms, depending on the binding partner, is one characteristic of intrinsically disordered proteins. The advantage of intrinsically disordered structures would be conserved in the Hef/FANCM proteins, which is consistent with computational predictions (42, 43). The IDR are also characterized by higher sequence divergence, as compared with the structural domains. The structural domains are well conserved through evolution, and can be detected by homology searches even across different domains of life (44, 45). By contrast, the IDR generally mutate more rapidly than the structural regions, presumably because the IDR are not structurally constrained against frequent insertions/deletions and amino acid substitutions. Indeed, the cases of the Hef/FANCM proteins revealed that the inter-domain regions are highly divergent, in contrast to the two distinct domains. All of our structural analyses presented in this study showed that the inter-domain regions of TkoHef are disordered in solution. Furthermore, we identified two proteins, PCNA1 and HAN, as binding partners of TkoHef at its IDR. We only present here that PCNA1 and HAN do not bind Hef-ID simultaneously from the experimental data showing failure to detect a ternary complex of Hef-ID:PCNA1-HAN, and the binding mode for each partner remains to be determined. We predict that a part or the entire region (104 amino acids long) of Hef-ID will be flexibly folded depending on its binding partners. The adoption of different structures will express the binding specificity to each partner. Although the sequences are divergent among species, their disordered structures would be conserved in the Hef/FANCM proteins.

**PCNA and HAN (a RecJ-like Protein) Do Not Bind Hef-ID Simultaneously**—The advantage of intrinsically disordered proteins is that they adopt various forms, depending on the binding partner. We expected that Hef-ID would fold in different manners for binding to PCNA1 and HAN. To investigate whether the IDR binds to PCNA1 and HAN simultaneously, we performed an SPR analysis. His-ID was loaded onto a HAN-immobilized sensor chip. As shown in Fig. 11B, His-ID bound the immobilized HAN in the opposite combination of ligand and analyte as the case of Fig. 11A. Addition of PCNA1 decreased the responses in the concentration-dependent manner (Fig. 11B). Furthermore, in the case of loading of His-ID onto a PCNA1-immobilized sensor chip, addition of HAN decreased the responses as well (Fig. 11C). The results of these experiments support the idea that different ligands could bind to the same region in the IDR.

**DISCUSSION**

Presence of IDR in the Hef/FANCM Proteins—Structural and functional analyses of human FANCM and its related proteins have become extremely popular, since FANCM was found to be the ortholog of the archaeal Hef protein. We focused here on the region between the N-terminal DEAH helicase-like domain and the C-terminal XPF/Mus81 nuclease-like domain of the Hef/FANCM proteins, because of their disordered structures. In general, IDR have a characteristically skewed amino acid composition, with an abundance of hydrophilic and charged residues, and few hydrophobic residues (42, 43). The IDR are also characterized by higher sequence divergence, as compared with the structural domains. The structural domains are well conserved through evolution, and can be detected by homology searches even across different domains of life (44, 45). By contrast, the IDR generally mutate more rapidly than the structural regions, presumably because the IDR are not structurally constrained against frequent insertions/deletions and amino acid substitutions. Indeed, the cases of the Hef/FANCM proteins revealed that the inter-domain regions are highly divergent, in contrast to the two distinct domains. All of our structural analyses presented in this study showed that the inter-domain region in TkoHef is disordered in solution. Furthermore, we identified two proteins, PCNA1 and HAN, as binding partners of TkoHef at its IDR. We only present here that PCNA1 and HAN do not bind Hef-ID simultaneously from the experimental data showing failure to detect a ternary complex of Hef-ID:PCNA1-HAN, and the binding mode for each partner remains to be determined. We predict that a part or the entire region (104 amino acids long) of Hef-ID will be flexibly folded depending on its binding partners. The adoption of different structures will express the binding specificity to each partner. Although the sequences are divergent among species, their disordered structures would be conserved in the Hef/FANCM proteins.

**FANC-interacting Proteins**—Human FANC interacts with many proteins to form an FA core complex (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, FAAP100, FAAP24, and HES1) on the stalled replication fork. The inter-domain region of human FANCM (1,243 amino acids) is 10 times longer than that in *T. kodakarensis*. The entire disorder of this inter-domain region can be predicted from its amino acid sequence, and it suggests chances to interact with much more proteins to process a more complex repair system in Eukarya as compared with Archaea. In the vertebrate homologs of FANCM, three highly conserved sequence motifs...
were identified in the inter-domain region and designated as MM1, MM2, and MM3 (FANCM motifs 1–3), and these motifs were predicted to be important for protein–protein interactions (46). Biochemical analyses revealed that MM1 interacts with the FA core complex by binding to FANCF, whereas MM2 interacts with RM1 and topoisomerase IIIa, components of the Bloom’s syndrome complex. Furthermore, MM1 and MM2 were independently required to activate the FA and Bloom’s syndrome repair pathways. Therefore, the proper function of FANCM is responsible for preventing both Fanconi anemia and Bloom’s syndrome. In addition, two histone-fold containing proteins, MHF1 and MHF2, which were recently identified as FANCM-associated factors, form the stable complex MHF1-MHF2 and stimulate the DNA-binding activity of FANCM (47, 48). The stable association with FANCM and the DNA-binding activity of MHF activate the FA repair pathway. The binding site of the MHF1-MHF2 complex, located between the DHAF helicase domain and MM1 (the region composed of amino acids 661–800) in FANCM is also predicted to be disordered (48). The crystal structure of an MHF1–MHF2–FANCM fragment was published quite recently (49). These findings indicated that the inter-domain region in FANCM is important for the interactions with several proteins in processing to the downstream reactions in the repair pathways, and the disordered structure of this region is suitable for accomplishing multiple functions of FANCM with its various interactive partners.

**PCNA Interacts with Hef**—We predicted that PCNA may interact with Hef, because of its function at the replication fork. Indeed, one of the two PCNA proteins in *T. kodakarenensis* bound to Hef at IDR. The bound PCNA was PCNA1, which is essential for the viability of *T. kodakarenensis* and is probably a regular member of the replisome (35). PCNA1 in the progressing replisome can stimulate recruitment of Hef when the replication fork stalls. A recent report describing the in vivo network analyses of the *Pyrococcus abyssi* cells showed that the Hef homolog is included in a His-tagged PCNA-bound fraction (50). This result also supports the interaction between PCNA and Hef in Archaea. From our experience, interaction with the PCNA protomer is not easy to detect by Y2H methods for an unknown reason, and even a homologous interaction of PCNA-PCNA (for its ring formation) cannot be detected. Therefore, the fact that the positive clones did not include PCNA from the Y2H experiment in this study is understandable. In *T. kodakarenensis*, PCNA1 is essential and the major clamp molecule in DNA replication. On the other hand, the gene for PCNA2 can be knocked out and the physiological function of PCNA2 has not been elucidated. Furthermore, many more molecules of PCNA1 exist as compared with PCNA2 (35). We analyzed only PCNA1 in this study, and will see the relationships between PCNA2 and Hef in our further works. In Eukarya, post-translational modifications of PCNA, including phosphorylation, acetylation, ubiquitination, and SUMOylation (51), regulate its binding to interacting proteins. However, neither ubiquitination nor SUMOylation have been reported in Archaea, and it is not clear if any post-translational modifications of PCNA are involved in the Hef-related repair process.

**Identification of HAN, a RecJ-like Nuclease, as a Hef-interacting Protein**—We obtained eight proteins as TkoHef-interacting proteins by the Y2H screening. Among these, HAN, a RecJ-like protein, is interesting, because it displayed an exonuclease activity. The concrete role of exonuclease activity of this protein remains to be investigated, but it should act downstream of incision at the stalled fork to start homologous recombination or remove lesions by a nucleotide excision repair-like process. It is interesting that two RecJ-like proteins are present in *T. kodakarenensis* and other archaeal organisms. One of them interacts with Hef (in this study) and the other interacts with GINS, an essential factor for DNA replication initiation and elongation in Eukarya and Archaea, which may be a component of the replisome helicase complex. The latter RecJ-like protein was reported as the GINS-associated nuclease (GAN), and may be involved in lagging strand processing (52). Furthermore, characterization of the GAN ortholog from *P. furiosus* revealed the 3′→5′ exonuclease activity, which may function in proof-reading for primer synthesis (53). The work sharing the two RecJ-like proteins, GAN and HAN, in Archaea is an interesting subject for future analyses.

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**REFERENCES**

Intrinsically Disordered Region of Hef/FANCM

31. Pluchon, P. F., Fouqueau, T., Creuzé, C., Laurent, S., Briffauta, J., Hogrel,


Multiple Interactions of the Intrinsically Disordered Region between the Helicase and Nuclease Domains of the Archaeal Hef Protein
Sonoko Ishino, Takeshi Yamagami, Makoto Kitamura, Noriyuki Kodera, Tetsuya Mori, Shyogo Sugiyama, Toshio Ando, Natsuko Goda, Takeshi Tenno, Hidekazu Hiroaki and Yoshizumi Ishino

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