Mutational Analysis of the *Pyrococcus furiosus* Holliday Junction Resolvase Hjc Revealed Functionally Important Residues for Dimer Formation, Junction DNA Binding, and Cleavage Activities

Received for publication, July 17, 2000, and in revised form, September 20, 2000
Published, JBC Papers in Press, December 25, 2000
DOI 10.1074/jbc.M006294200

Kayoko Komori‡, Shinzi Sakae‡‡, Hiromi Daiyasu‡, Hiroyuki Toh‡, Kosuke Morikawa‡, Hideo Shinagawa§, and Yoshizumi Ishino‡**

From the Departments of ‡Molecular Biology, ‡Bioinformatics, and §Structural Biology, Biomolecular Engineering Research Institute, Suita, Osaka 565-0874 and the ‡Department of Molecular Microbiology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565-0874, Japan

The Holliday junction cleavage protein, Hjc resolvase of *Pyrococcus furiosus*, is the first Holliday junction resolvase to be discovered in Archaea. Although the archaean resolvase shares certain biochemical properties with other non-archaean junction resolvases, no amino acid sequence similarity has been identified. To investigate the structure-function relationship of this new Holliday junction resolvase, we constructed a series of mutant hjc genes using site-directed mutagenesis targeted at the residues conserved among the archaean orthologs. The products of these mutant genes were purified to homogeneity. With analysis of the activity of the mutant proteins to bind and cleave synthetic Holliday junctions, one acidic residue, Glu-9, and two basic residues, Arg-10 and Arg-25, were found to play critical roles in enzyme action. This is in addition to the three conserved residues, Asp-33, Glu-46, and Lys-48, which are also conserved in the motif found in the type II restriction endonuclease family proteins. Two aromatic residues, Phe-68 and Phe-72, are important for the formation of the homodimer probably through hydrophobic interactions. The results of these studies have provided insights into the structure-function relationships of the archaean Holliday junction resolvase as well as the universality and diversity of the Holliday junction cleavage reaction.

Homologous DNA recombination is one of the most extensively studied subjects in the field of DNA transactions (reviewed in Refs. 1 and 2). The molecular mechanisms of the early stages of homologous recombination from the introduction of double strand breaks to the formation of recombination intermediates in which two homologous duplex DNA molecules are held together by a single-stranded crossover have been studied extensively (3, 4). Although several studies in eukaryotic systems have been recently reported since the identification of Rad51, the structural and functional homolog of RecA (5, 6), the mechanism of late-stage DNA recombination, in which the Holliday junctions are processed correctly into the recombinant duplexes, have thus far been analyzed mostly in *Escherichia coli* (7, 8). There, the RuvA and RuvB protein complex specifically binds to the Holliday junction and promotes branch migration. RuvC protein cleaves the Holliday junction when the junction migrates to the favored sequence for the cleavage. In addition to biochemical properties, the crystal structure of RuvC endonuclease was determined at 2.5 Å resolution, and a putative model of RuvC complexed with the Holliday junction was proposed, suggesting a cleavage mechanism at the junction DNA (9, 10).

The Holliday junction resolving activities have been identified in a wide variety of organisms, from bacteriophage to mammals (reviewed in Refs. 11 and 12). However, the corresponding enzymes were isolated only from bacteriophage T4 (endonuclease VII), bacteriophage T7 (Endonuclease I), lambia prophage (RusA), and yeast mitochondria (endonucleases CCE1 and SpCCE) besides *E. coli* RuvC. No open-reading frames with sequences similar to that of the bacterial RuvA, RuvB, and RuvC proteins in Eukarya and Archaea were discovered in the current public databases, including the total genomes of *Saccharomyces cerevisiae* (13) and five archaeal strains, *Methanococcus jannaschii* (14), *Archaeoglobus fulgidus* (15), *Methanobacterium thermoautotrophicum* (16), *Pyrococcus horikoshii* (17), and *Aeropyrum pernix* (18).

We identified a Holliday junction cleaving activity in the hyperthermophilic archaean, *Pyrococcus furiosus* and cloned the gene encoding this Holliday junction cleavage protein (Hjc, Ref. 19). Further biochemical and physical properties of the purified Hjc have been investigated (20), and more recently, the Hjc homolog in *Sulfolobus solfataricus* has been reported by Kvaratskhelia and White (21). Although the amino acid sequences of archaeal Hjc proteins are highly conserved (19, 21), they are not similar to junction resolvases of other domains. Hjc proteins do however share some local sequence similarities to the type II restriction endonucleases, and therefore, we have proposed that they are related to the type II restriction endonuclease family (22).

To investigate the structure-function relationship of this new Holliday junction resolvase, we analyzed the biochemical properties of 14 mutant Hjc proteins that were produced by site-directed mutagenesis. From these analyses, we identified several amino acid residues, each of which is critical for substrate binding, catalysis, and dimer formation.

MATERIALS AND METHODS

DNA Substrates—The four-way junctions, 4Jh with a 12-base pair homologous core (for the cleavage assay) and 4Jb (for the gel retardation assay) were generated by using the MADIGENsite-directed mutagenesis kit (Takara). A 12-base pair homologous core is located between perfect 12-base pair sequences, and four perfect 12-base pairs are flanked by two 6-base pairs, respectively.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

** To whom correspondence should be addressed: Dept. of Molecular Biology, Biomolecular Engineering Research Inst., 6-2-3 Furuedai, Suita, Osaka 565-0874, Japan. Tel: 81-6-6872-8208; Fax: 81-6-6872-8219; E-mail: ishino@beri.co.jp.
RESULTS

Amino Acid Sequence Comparison of Archaeal Hjc Proteins—To select the putative important residues for the Holliday junction cleavage activity of Hjc, we examined the multiple
sequence alignment of the archaeal Hjc proteins found to date (Fig. 1). The molecular sizes and sequences (especially the N-terminal half-region) of the archaeal Hjc are highly conserved. Several nucleases that require a divalent cation for catalysis have 3 or 4 acidic residues constituting the catalytic centers of these enzymes (29–36). Four residues, Asp-7, Glu-66, Asp-138, and Asp-141 of *E. coli* RuvC, for example, are known to be essential for resolvase activity but not for dimer formation and binding to the Holliday junction (37). A crystallographic study showed that the four residues are located in close proximity to each other at the bottom of a putative DNA-binding cleft (9). These results suggest that the residues constitute the catalytic center of RuvC and coordinate Mg$^{2+}$ and water for hydrolysis. The sequence alignment of five archaeal homologs show some conservation of the acidic residues (Glu-9, Glu-11, Asp-33, Glu-46, and Glu-110 in Hjc), which may include the catalytic center of this enzyme. We have previously proposed that some of these residues are within a motif conserved in the type II restriction endonucleases (22). Thus, we targeted the conserved residues, including the acidic residues, for mutagenesis. A total of 11 residues were selected and subjected to site-directed mutagenesis, in addition to the three residues identified in our previous work (22). When we started this work, the two crenarchaeal sequences (from *A. pernix* and *S. solfataricus*) were not available, and therefore, some of the target residues were not conserved in all sequences (Fig. 1).

**Preparation of Mutant Hjc Proteins**—Mutant *hjc* genes were expressed in *E. coli* BL21 (DE3) using the pET21a vector, similar to wild-type Hjc. The production levels of the mutant proteins in the cells were the same as that of wild-type Hjc. All of the mutant proteins exhibited the same purification behavior as the wild-type proteins, which suggested that the mutant proteins maintained the original Hjc conformation. After hydroxyapatite column chromatography, the Hjc proteins were detected as a clear single band by SDS-PAGE followed by Coomassie Brilliant Blue staining (Fig. 2), and therefore the Mono Q anion-exchange and heparin-Sepharose affinity chromatography steps, which we used for the purification of wild-type Hjc (19), were omitted. About 3 mg of protein on average were obtained from 200 ml of culture.

**Cleavage Activities of Mutant Hjc Proteins**—All mutant proteins were assayed for the ability to cleave the Holliday junction. As shown in Fig. 3, more than 90% of the substrate (100 nM) was cleaved by 10 nM wild-type Hjc. Under the same conditions, no cleaved product was detected from nine mutant proteins, E9A, R10A, R25A, D33A, E46A, K48A, F68A, F72A, and K81A. Some decrease in the activity was observed for the Y56A mutant protein. To measure the residual activities more precisely, the reactions were performed with higher concentrations of the proteins, and the activities relative to that of wild-type Hjc were calculated (Table II). In this experiment, an interesting property of F72A was apparent. The F72A protein cleaved near the junction center to produce two duplex DNAs as observed in the normal Hjc cleavage activity at concentrations of less than 400 nM. However, F72A cleaved other sites to produce smaller fragments with increasing concentrations (1–4 μM) of the protein in the reaction mixture (Fig. 3B). This unique cleavage activity may be attributed to the F72A protein rather than contaminated nucleases, because this activity was not detected with the same concentrations of wild-type Hjc (Fig. 3B) or other mutant proteins (data not shown) purified by the same procedure as described above. As shown in Fig. 4,
F72A showed the same cleavage pattern as that of the wild-type, which cleaves near the junction center (mostly between the third and fourth nucleotides measured from the 3'-side of the junction). Additionally, F72A cleaved the junction DNA at several positions in the middle of the arms. These extra cleavage sites occurred only in one particular strand of the duplex DNA. Under the high F72A concentration conditions, normal duplex DNAs containing the same oligonucleotide sequence as the Holliday junction were also cleaved with the same cleavage patterns (Fig. 4A). The single-stranded DNAs were not cleaved under the same conditions (Fig. 4A).

### Table II

| Mutation | Relative activity |
|----------|-------------------|
| wt       | 1                 |
| E9A      | <0.01             |
| R10A     | 0.01              |
| E11A     | 1                 |
| F21A     | 1                 |
| R25A     | <0.01             |
| D33A     | <0.01             |
| E46A     | <0.01             |
| K48A     | <0.01             |
| Y56A     | 0.7               |
| F68A     | 0.02              |
| F72A     | <0.01             |
| K81A     | <0.01             |
| F89A     | 1                 |
| E110A    | 1                 |

**Binding of Hjc Mutant Proteins to the Holliday Junction**—To analyze the cause of decreased cleavage activity of the mutant Hjcs, the substrate binding activity was measured by a gel retardation assay using a 32P-labeled Holliday junction. As shown in Fig. 5, the intensity of the discretely retarded bands attributed to junction DNA-Hjc complex formation increased with higher protein concentrations for most of the mutant proteins. Some decrease in the formation of the complex band was observed for K81A. A slight decrease of the complex band was also observed for E110A. However, this may be an experimental artifact because E110A protein has cleavage activity that is as strong as that of the wild-type Hjc as shown above. In addition, remarkably different patterns were observed in the reactions with F68A and F72A in which two shifted bands were

**Fig. 4. Determination of the secondary cleavage sites produced by the F72A mutant protein.** A, the mobile four-way junction 4Jh (lanes 1 and 2), 32P-labeled uniquely at the 5'-end of each indicated strand (*), was used as the substrate. A duplex DNA (lanes 3-5) and a single-stranded DNA (lanes 6-8) with a 32P-labeled arm of a 4Jh were also used. A 4-μM concentration of the wild-type protein (lanes 1, 4, and 7) or the F72A protein (lanes 2, 5, and 8) was reacted with 0.1 μM DNA substrates at 56 °C for 30 min. The products were separated on a 9% polyacrylamide gel containing 8M urea and were detected by autoradiography. B, open arrowheads show the cleavage sites in the junction generated by both the wild-type and F72A proteins. Closed arrowheads indicate the sites that uniquely appeared with the F72A protein. The cleavage efficiencies are denoted by the sizes of the arrowheads. The boxed region of the junctions indicates the mobile homologous sequence.
clearly detected. Because Hjc exists as a homodimer in solution (19) and binds to the Holliday junction in the dimerized form (20), we hypothesized that the two bands shifted by F68A and F72A reflected the formation of Hjc monomer-junction DNA and Hjc dimer-junction DNA, respectively.

**Dimer Formation by Mutant Hjc Proteins**—The dimer forming ability of each mutant protein was investigated by two different methods, chemical cross-linking and sedimentation equilibrium analysis. Hjc mutant proteins were treated with glutaraldehyde and were fractionated by SDS-PAGE. The Coomassie Brilliant Blue stained gel showed that the band corresponding to 28 kDa, in addition to the one at 14 kDa, was detected with all the mutant and wild-type proteins although the proportions of the two bands varied (data not shown). This result indicates that glutaraldehyde covalently linked the dimer form of the proteins, thus generating the 28-kDa band on the SDS-PAGE. In this experiment, F68A and F72A gave distinctively faint bands of the dimer form. Each band on the stained gel was quantified by using an image analyzer, and the cross-linking efficiency was calculated for each of the mutant proteins (Fig. 6A). To confirm the result that the mutations of F68 and F72 affected the dimer-forming ability of Hjc, the sedimentation equilibrium profile of each mutant protein was measured using an analytical ultracentrifuge. From the obtained average molecular weights, the association constant, $K_a$, was calculated for each protein. Based on these data, the ratio of dimer/monomer in the total molecules were calculated (Fig. 6B). The result clearly shows that the F68A and F72A mutant proteins exist as monomers more frequently in solution than as dimers.

**DISCUSSION**

Hjc is a newly discovered junction resolvase recently found in Archaea. The amino acid sequence of Hjc is not similar to any of the known junction resolvases in other biological domains, even though it shares many common biochemical properties. The present mutational analysis of Hjc provides important information about the structure-function relationship of Hjc necessary for the resolution of the Holliday junction.

Database searching with PSI-BLAST detected 59 amino acid sequences similar to Hjc resolvase (22). The detected sequences include the three members of the type II restriction endonuclease family, DpnII, HaeII, and the Var endonuclease, which contain a highly conserved pattern, Asp-X$_8$-(Asp/Glu)-hydrophobic residue-Lys (22). We substituted each of the three conserved residues in this motif of the Hjc resolvase with Ala and found that all of the substitutions inactivated the enzyme. This observation suggests that the archaeal Hjc resolvases belong to the type II restriction endonuclease family (22). There are many type II restriction endonucleases, with solved tertiary structures (reviewed in Ref. 38). However, of the 59 sequences detected by our database search, the _E. coli_ Var endonuclease (26, 39) was the only protein whose coordinates were available. The x-ray crystallographic analysis revealed that the Var endonuclease shares its folding pattern with the type II restriction endonucleases (26), although this enzyme does not function as a restriction enzyme, but is involved in the repair of TG mismatches within DNA. We generated a manual alignment between the _P. furiosus_ Hjc resolvase and the Var endonuclease (Fig. 7). The matching of two predicted helices to the actual secondary structures of Var endonuclease (alignment sites 88–101 and 139–144) and that of a predicted strand to the corresponding secondary structure of Var endonuclease (alignment sites 156–165) differed from the ClustalW output, because we slightly shifted the positions by visual inspection to increase the residue matching to the actual secondary structures of Var endonuclease. Other aligned regions were kept as the ClustalW output. As shown in Fig. 7, the predicted secondary structures of Hjc matched the actual Var structure very well.

From the cleavage assays in this study, major decreases in the activity were observed in 9 mutants, E9A, R10A, R25A, F68A, F72A, and K81A, in addition to the three conserved residues (D33A, E46A, and K48A) in the restriction endonuclease family, _HaeII_, _DpnII_, and the _Var_ endonuclease, which contain a highly conserved pattern, Asp-X$_8$-(Asp/Glu)-hydrophobic residue-Lys (22). We substituted each of the three conserved residues in this motif of the Hjc resolvase with Ala and found that all of the substitutions inactivated the enzyme. This observation suggests that the archaeal Hjc resolvases belong to the type II restriction endonuclease family (22). There are many type II restriction endonucleases, with solved tertiary structures (reviewed in Ref. 38). However, of the 59 sequences detected by our database search, the _E. coli_ Var endonuclease (26, 39) was the only protein whose coordinates were available. The x-ray crystallographic analysis revealed that the Var endonuclease shares its folding pattern with the type II restriction endonucleases (26), although this enzyme does not function as a restriction enzyme, but is involved in the repair of TG mismatches within DNA. We generated a manual alignment between the _P. furiosus_ Hjc resolvase and the Var endonuclease (Fig. 7). The matching of two predicted helices to the actual secondary structures of Var endonuclease (alignment sites 88–101 and 139–144) and that of a predicted strand to the corresponding secondary structure of Var endonuclease (alignment sites 156–165) differed from the ClustalW output, because we slightly shifted the positions by visual inspection to increase the residue matching to the actual secondary structures of Var endonuclease. Other aligned regions were kept as the ClustalW output. As shown in Fig. 7, the predicted secondary structures of Hjc matched the actual Var structure very well.

From the cleavage assays in this study, major decreases in the activity were observed in 9 mutants, E9A, R10A, R25A, F68A, F72A, and K81A, in addition to the three conserved residues (D33A, E46A, and K48A) in the restriction endonuclease family, _HaeII_, _DpnII_, and the _Var_ endonuclease, which contain a highly conserved pattern, Asp-X$_8$-(Asp/Glu)-hydrophobic residue-Lys (22). We substituted each of the three conserved residues in this motif of the Hjc resolvase with Ala and found that all of the substitutions inactivated the enzyme. This observation suggests that the archaeal Hjc resolvases belong to the type II restriction endonuclease family (22). There are many type II restriction endonucleases, with solved tertiary structures (reviewed in Ref. 38). However, of the 59 sequences detected by our database search, the _E. coli_ Var endonuclease (26, 39) was the only protein whose coordinates were available. The x-ray crystallographic analysis revealed that the Var endonuclease shares its folding pattern with the type II restriction endonucleases (26), although this enzyme does not function as a restriction enzyme, but is involved in the repair of TG mismatches within DNA. We generated a manual alignment between the _P. furiosus_ Hjc resolvase and the Var endonuclease (Fig. 7). The matching of two predicted helices to the actual secondary structures of Var endonuclease (alignment sites 88–101 and 139–144) and that of a predicted strand to the corresponding secondary structure of Var endonuclease (alignment sites 156–165) differed from the ClustalW output, because we slightly shifted the positions by visual inspection to increase the residue matching to the actual secondary structures of Var endonuclease. Other aligned regions were kept as the ClustalW output. As shown in Fig. 7, the predicted secondary structures of Hjc matched the actual Var structure very well.

From the cleavage assays in this study, major decreases in the activity were observed in 9 mutants, E9A, R10A, R25A, F68A, F72A, and K81A, in addition to the three conserved residues (D33A, E46A, and K48A) in the restriction endonuclease family, _HaeII_, _DpnII_, and the _Var_ endonuclease, which contain a highly conserved pattern, Asp-X$_8$-(Asp/Glu)-hydrophobic residue-Lys (22). We substituted each of the three conserved residues in this motif of the Hjc resolvase with Ala and found that all of the substitutions inactivated the enzyme. This observation suggests that the archaeal Hjc resolvases belong to the type II restriction endonuclease family (22). There are many type II restriction endonucleases, with solved tertiary structures (reviewed in Ref. 38). However, of the 59 sequences detected by our database search, the _E. coli_ Var endonuclease (26, 39) was the only protein whose coordinates were available. The x-ray crystallographic analysis revealed that the Var endonuclease shares its folding pattern with the type II restriction endonucleases (26), although this enzyme does not function as a restriction enzyme, but is involved in the repair of TG mismatches within DNA. We generated a manual alignment between the _P. furiosus_ Hjc resolvase and the Var endonuclease (Fig. 7). The matching of two predicted helices to the actual secondary structures of Var endonuclease (alignment sites 88–101 and 139–144) and that of a predicted strand to the corresponding secondary structure of Var endonuclease (alignment sites 156–165) differed from the ClustalW output, because we slightly shifted the positions by visual inspection to increase the residue matching to the actual secondary structures of Var endonuclease. Other aligned regions were kept as the ClustalW output. As shown in Fig. 7, the predicted secondary structures of Hjc matched the actual Var structure very well.
ase family (Fig. 3 and Table II). The binding assays showed a slightly decreased binding activity for the Holliday junction by K81A and E110A (Fig. 5), and F68A and F72A are deficient in dimer-forming ability (Fig. 6). From these analyses, it can be predicted that Glu-9, Arg-10, Arg-25, Asp-33, Glu-46, and Lys-48 are directly involved in the catalytic mechanism of the junction cleavage reaction by Hjc. In the three-dimensional structure model of Hjc, which was built by using the structure of Vsr protein as the template, Glu-9, Arg-10, and Arg-25 can be located spatially close to the three putative active residues described above (data not shown). However, further analyses are necessary to understand the role of these residues in the cleavage reaction.

The two hydrophobic residues, Phe-68 and Phe-72, of Hjc seem to be involved in the dimer formation of the enzyme. The crystal structures of RuvC and T4 endonuclease VII reveal that the dimer formation by these junction resolvases occurs by inter-subunit interactions, mainly between the two α-helices, which are arranged in parallel to the dyad axis in the dimer (9, 40). The two hydrophobic residues, Phe-68 and Phe-72, are located in the same α-helix among the predicted secondary structures of Hjc (Fig. 7), and the predicted α-helix may be involved in the interactions between subunits. One more interesting finding in regard to the Phe-72 residue is that the F72A mutant protein has an unique property. The normal duplex DNA can be cleaved by a high concentration of F72A but not by any of the other proteins, including wild-type Hjc (Figs. 3 and 4). This change is not caused by a defect in dimer formation, because the F68A protein never showed the same cleavage pattern as that of F72A, even though they both have similar deficiencies in dimer formation. These results suggest that Phe-72 may play a very important role in recognizing the specific structure of DNA strands.

Mutations altering the two residues, Lys-81 and Glu-110 reduced the DNA binding. The binding mode of the enzyme to the Holliday junction remains unknown at this stage. These residues might have a cumulative effect on the junction binding or these mutations might alter the local conformation of the enzyme resulting in reduction of the binding activity.

During the review process of this work, a mutational study of S. solfataricus Hjc, which supports our finding that Hjc belongs to the restriction endonuclease family was published by White and co-workers (41), and the structural relationships of the junction resolvases were discussed (42). Further mutational analyses of Hjc, combined with crystallographic studies, should give greater insights into the structure-function relationships of the Hjc junction resolvase. These studies will also reveal universality and diversity of the Holliday junction cleavage reactions.

Acknowledgments—We thank Drs. M. Shimizu and T. Hiroike for help with the analytical ultracentrifugaf and structure prediction, respectively. We are grateful to Dr. Y. Shimura, the director of BERI, for continuous encouragement.

REFERENCES

1. Kowalczykowski, S. C., Dixon, D. A., Egleston, A. K., Lauder, S. D., and Rehrauer, W. M. (1994) Microbiol. Rev. 58, 401–465
2. Shinohara, A., and Ogawa, T. (1992) Proc. Natl. Acad. Sci. USA 89, 6905–6909
3. Shinohara, A., Ogawa, T. (1995) in DNA-protein: Structural Interactions (Lilley, D. M., ed), Vol. 12, pp. 275–299, Springer-Verlag, Berlin Heidelberg
4. Bianco, P. R., Tracy, R. B., and Kowalczykowski, S. C. (1998) Trends Biochem. Sci. 23, 113–117
5. Shinohara, A., Ogawa, H., and Iwasaki, H. (1998) Annu. Rev. Genet. 32, 107–111
6. West, S. C. (1997) Annu. Rev. Biochem. 66, 177–202
7. Shinagawa, H., and Iwasaki, H. (1996) Trends Biochem. Sci. 21, 22–29
White, M. F., Giraud-Panis, M.-J. E., Pohler, J. R. G., and Lilley, D. M. J. (1997) J. Mol. Biol. 269, 647–664.

Goffeau, A., Barrell, B. G., Bussey, H., Davis, R. W., Dujon, B., Feldmann, H. Galibert, F., Hoheisel, J. D., Jacq, C., Johnston, M., Louie, E. J., Mewes, H. W., Murakami, Y., Philippsen, P., Tettelin, H., and Oliver, S. G. (1996) Science 274, 546–560.

Bult, C. J., White, O., Olsen, G. J., Zhou, L., Fleischmann, R. D., Adams, M. D., Reich, C. I., Overbeek, R., Kirkness, E. F., Weinstock, K. G., Merrick, J. M., Gloyd, A., Scott, J. L., Geoghegan, N. M. S., Weidman, J. F., Fuhrmann, J. L., Presley, K. A., Nguyen, D., Utterback, T. R., Kelley, J. M., Peterson, J. D., Sadow, P. W., Hanna, M. C., Cotton, M. D., Hurst, M. A., Roberts, K. M., Kaine, B. P., Borodovsky, M., Klenk, H.-P., Fraser, C. M., Smith, H. O., Woese, C. R., and Venter, J. C. (1996) Science 273, 1058–1073.

Klenk, H.-P., Clayton, R. A., Tesh, J., White, O., Nelson, K. E., Ketchum, K. A., Dodson, R. J., Gwinn, M., Hickey, E. K., Peterson, J. D., Richardson, D. L., Kerlavage, A. R., Graham, D. E., Kyrpides, N. C., Fleischmann, R. D., Quackenbush, J., Lee, N. H., Sutton, G. G., Gill, S., Kirkness, E. F., Dougherty, B. A., McKenney, K., Adams, M. D., Loftus, B., Petersen, S., Reich, C. I., McNeil, L. K., Badger, J. H., Gloyd, A., Zhou, L., Overbeek, R., Gocayne, J. D., Weidman, J. F., McDonald, L., Utterback, T., Cotton, M. D., Spriggs, T., Artiach, P., Kaine, B. P., Sykes, S. M., Sadow, P. W., D'Andrea, K. P., Bowman, C., Fujii, C., Garland, S. A., Mason, T. M., Olsen, G. J., Fraser, C. M., Smith, H. C., Woese, C. R., and Venter, J. C. (1997) Nature 390, 264–270.

Smith, D. R., Doucette-Stamm, I. A., Delougey, C., Lee, H.-M., Dubois, J. A., Aldredge, T., Bashirzadeh, R., Blakely, D., Cook, R., Gilbert, K., Harrison, D., Hoang, L., Keagle, P., Lumm, W., Peterson, S., Pothier, B., Qu, D., Spadafora, R., Vicare, R., Wang, Y., Wierzchowski, J., Gibson, R., Jiawan, C., Nishida, S., Caruso, A., Bush, D., Safer, H., Patwell, D., Prabhakar, S., McDougall, S., Shimny, G., Goyal, A., Pietrotti, Y., Church, G. M., Daniels, C. L., Mao, J.-I., Rice, P., Neiling, J., and Reeve, J. N. (1997) J. Bacteriol. 179, 7135–7155.

Kawarabayasi, Y., Sawada, M., Horikawa, H., Haikawa, H., Ariyoshi, M., Yamamoto, S., Sekine, M., Baba, S., Kosugi, H., Hosoya, A., Nagai, Y., Sakai, M., Ouchi, K., Osako, H., Nakazawa, H., Takamiya, M., Aoki, A., Funahashi, T., Tanaka, T., Kudoh, Y., Yamazaki, K., Kudoh, S., Oguchi, A., Aoki, K., Yoshizawa, T., Nakamura, Y., Bobb, F. T., Horikoshi, K., Masuchi, Y., Shizuya, H., Fujita, M., and Kikuchi, H. (1998) DNA Res. 5, 55–76.

Kawarabayasi, Y., Hino, Y., Horikawa, H., Yamazaki, S., Haikawa, Y., Jin-no, K., Takahashi, M., Sekine, M., Baba, S., Ankai, A., Kosugi, H., Hosoya, A., Fukui, S., Nagai, Y., Nishijima, K., Nakazawa, H., Takamiya, M., Masaeda, S., Funahashi, T., Tanaka, T., Kudoh, Y., Yamazaki, K., Kushida, N., Oguchi, A., and Kikuchi, H. (1999) DNA Res. 6, 83–101.

19. Komori, K., Sakae, S., Shinagawa, H., Morikawa, K., and Ishino, Y. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 8873–8878.

20. Komori, K., Sakae, S., Fujikane, R., Shinagawa, H., Morikawa, K., and Ishino, Y. (2000) Nucleic Acids Res. 28, 4544–4551.

21. Komori, K., Sakae, S., Fujikane, R., Shinagawa, H., Morikawa, K., and Ishino, Y. (2000) Nucleic Acids Res. 28, 2645–2651.

22. Komori, K., Sakae, S., Fujikane, R., Shinagawa, H., Morikawa, K., and Ishino, Y. (2000) Nucleic Acids Res. 28, 2645–2651.

23. Komori, K., Sakae, S., Fujikane, R., Shinagawa, H., Morikawa, K., and Ishino, Y. (2000) Nucleic Acids Res. 28, 2645–2651.

24. Komori, K., Sakae, S., Fujikane, R., Shinagawa, H., Morikawa, K., and Ishino, Y. (2000) Nucleic Acids Res. 28, 2645–2651.

25. Komori, K., Sakae, S., Fujikane, R., Shinagawa, H., Morikawa, K., and Ishino, Y. (2000) Nucleic Acids Res. 28, 2645–2651.

26. Komori, K., Sakae, S., Fujikane, R., Shinagawa, H., Morikawa, K., and Ishino, Y. (2000) Nucleic Acids Res. 28, 2645–2651.

27. Cohn, E. J., and Edsall, J. T. (1943) Proteins, Amino acids and Peptides as Ions and Dipolar ions. pp. 370–381, Reinhold Publishing Corp., New York.

28. Rost, B., and Sander, C. (1994) Proteins, 55–77.

29. Schatz, O., Cremers, P. V. M., and Gruninger-Leitch, F., and Le Grice, S. F. J. (1999) FEBS Lett. 257, 311–314.

30. Kanaya, S., Kobara, H., Muray, Y., Sekiguchi, A., Iwai, S., Inoue, H., Ohatsuka, E., and Ikehara, M. (1999) J. Biol. Chem. 265, 4615–4621.

31. Mizrahi, V., Uusin, M. T., Harington, A., and Dudding, L. R. (1990) Nucleic Acids Res. 18, 5359–5363.

32. Davies, I. J., F. Hostomsky, Z., Hostomsky, Z., Jordan, S. R., and Matthews, D. A. (1991) Science 252, 88–95.

33. Katayanagi, K., Miyagawa, M., Matsushima, I., Ishikawa, M., Kanaya, S., Nakamura, H., Ichikawa, M., Matsuzaki, T., and Morikawa, K. (1992) J. Mol. Biol. 225, 1029–1052.

34. Katayanagi, K., Ishikawa, M., Okumura, M., Ariyoshi, M., Kanaya, S., Kawano, Y., Suzuki, M., Tanaka, I., and Morikawa, K. (1993) J. Biol. Chem. 268, 22992–22999.

35. Katayanagi, K., Ishikawa, M., Okumura, M., Ariyoshi, M., Kanaya, S., Kawano, Y., Suzuki, M., Tanaka, I., and Morikawa, K. (1993) J. Biol. Chem. 268, 22992–22999.

36. Joyce, C. M., and Steitz, T. A. (1994) Annu. Rev. Biochem. 63, 777–822.

37. Saito, A., Iwasaki, H., Ariyoshi, M., Morikawa, K., and Shinagawa, H. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 7470–7474.

38. Pingoud, A., and Jeltsch, A. (1997) Eur. J. Biochem. 246, 1–22.

39. Tsutakawa, S. E., Jingami, H., and Morikawa, K. (1999) Cell 99, 615–623.

40. Tsutakawa, S. E., Jingami, H., and Morikawa, K. (1999) Cell 99, 615–623.

41. Tsutakawa, S. E., Jingami, H., and Morikawa, K. (1999) Cell 99, 615–623.

42. Tsutakawa, S. E., Jingami, H., and Morikawa, K. (1999) Cell 99, 615–623.
Mutational Analysis of the Pyrococcus furiosus Holliday Junction Resolvase Hjc Revealed Functionally Important Residues for Dimer Formation, Junction DNA Binding, and Cleavage Activities

Kayoko Komori, Shinzi Sakae, Hiromi Daiyasu, Hiroyuki Toh, Kosuke Morikawa, Hideo Shinagawa and Yoshizumi Ishino

J. Biol. Chem. 2000, 275:40385-40391. doi: 10.1074/jbc.M006294200 originally published online September 25, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M006294200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 40 references, 12 of which can be accessed free at http://www.jbc.org/content/275/51/40385.full.html#ref-list-1