A Conserved Nuclease Domain in the Archaeal Holliday Junction Resolving Enzyme Hjc*

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Holliday junction resolving enzymes are ubiquitous proteins that function in the pathway of homologous recombination, catalyzing the rearrangement and repair of DNA. They are metal ion-dependent endonucleases with strong structural specificity for branched DNA species. Whereas the eukaryotic nuclear enzyme remains unknown, an archaeal Holliday junction resolving enzyme, Hjc, has recently been identified. We demonstrate that Hjc manipulates the global structure of the Holliday junction into a 2-fold symmetric X shape, with local disruption of base pairing around the point of cleavage that occurs in a region of duplex DNA 3' to the point of strand exchange. Primary and secondary structural analysis reveals the presence of a conserved catalytic metal ion binding domain in Hjc that has been identified previously in several restriction enzymes. The roles of catalytic residues conserved within this domain have been confirmed by site-directed mutagenesis. This is the first example of this domain in an archaeal enzyme of known function as well as the first in a Holliday junction resolving enzyme.

Holliday junction resolving enzymes play a role in the pathway of homologous recombination, recognizing and cleaving the four-way DNA junctions that arise from strand exchange between homologous duplex DNA species. Junction resolving enzymes are ubiquitous in nature. These proteins have been identified in Eubacteria (RuvC (1, 2) and RusA (3)), bacteriophage (T4 endonuclease VII (4) and T7 endonuclease I (5, 6)), fungal mitochondria (Cce1 (7)), and most recently Archaea (Hjc and Hje (8, 9)). Whereas activities have been detected in nuclear extract from yeast (10) and mammalian cells (11, 12), the relevant genes have yet to be identified. Resolving enzymes function as dimers, resolving the four-way DNA junction by the introduction of paired nicks in opposing strands with a magnesium-dependent endonuclease activity. Despite these functional similarities, the junction resolving enzymes are structurally diverse with no detectable sequence similarity among any of the known examples. Structural studies have highlighted this diversity because the crystal structures of RuvC (13), T4 endonuclease VII (14), and T7 endonuclease I (15) have radically different folds. These observations have led to the suggestion that resolving enzymes have arisen several times during the course of evolution, perhaps by recruitment of nucleases with other cellular roles. This is almost certainly the case for the eubacterial enzyme RuvC, which shares a fold and metal binding site with members of the RNase HI superfamily (13, 15).

The Archaea constitute a third domain of life that is distinct from both the Eubacteria and the Eucarya. Whereas the Archaea resemble their fellow prokaryotes in most respects, they share many similarities with the Eucarya in the information processing pathways including DNA replication, transcription, and translation (reviewed in Ref. 16), and the archaeal processes constitute a useful model system for the much more complex eucaryal equivalents. We are investigating the pathway of homologous recombination in the Archaea and have detected two Holliday junction resolving enzymes, Hje and Hjc, in the Crenarchaeote Sulfolobus solfataricus (8, 17). The gene for Hjc has been identified and is conserved in all Archaea for which extensive genome sequence is available (8, 9). We have cloned S. solfataricus Hjc and overexpressed the recombinant enzyme in Escherichia coli (8). Here we demonstrate manipulation of the global structure of the four-way junction substrate by Hjc and disruption of base stacking near the cleavage site. We report the identification of a structural motif in Hjc that is shared by a diverse group of nucleases and constitutes a binding site for the catalytic metal ions. Parallels between Holliday junction resolving enzymes and restriction enzymes that were first pointed out at a biochemical level for the yeast mitochondrial enzyme Cce1 (18) now achieve a firm structural basis in the archaeal Hjc enzyme.

MATERIALS AND METHODS

Oligonucleotide Synthesis and Assembly of DNA Junctions

Oligonucleotides were synthesized and four-way DNA junctions assembled as described previously (18) using the sequences described in the following paragraphs.

**Junction 3**—This fixed four-way junction was prepared with arms of 15 or 25 bp as described previously (45). Comparative gel electrophoresis experiments utilized a version of junction 3 with four arms of 60 bp in length. Six forms of junction 3 with two long and two short arms were derived from this junction as described previously (46).

**Junction 1**—This is a fixed junction with 20 bp in each arm assembled from four oligonucleotides, each of 40 nucleotides in length as described previously (47).

**Junction Z28**—This junction is utilized for permanganate-probing experiments. The junction has arms of 15 bp including eight thymine residues centered around the point of strand exchange on the r strand: b, 5'-TCCGTCCTAGCAAGAGCTGCTACCGGAA; h, 5'-TCCGTTAGCAGACATTTAAAGGTGTTGAAT; r, 5'-ATCAACACCTTTTATTAAGCTCAGCCAG; x, 5'-CTGCTGAGTTAAACCCCTTTACTGCTAGGACGGA.

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1 S. E. V. Phillips and D. M. J. Lilley, personal communication.

2 The abbreviation used is: bp, base pairs.
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Expression and Purification of Recombinant Hjc Protein

Recombinant Hjc protein was expressed in *E. coli* strain BL21 (DE3) CodonPlus RIL (Stratagene), and the protein was purified as described previously (8). In brief, Hjc was purified by chromatography on an SP-Sepharose high performance 26/10 column (Hi-Load, Amersham Pharmacia Biotech) equilibrated with Buffer A (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol). A 500-ml linear gradient of 0–1000 mM NaCl was used to elute cationic proteins. Fractions corresponding to a distinct absorbance peak were analyzed by SDS-polyacrylamide gel electrophoresis, pooled, concentrated, and loaded onto a 26/70 gel filtration column (Superdex 200 Hi-Load, Amersham Pharmacia Biotech) and developed with Buffer A containing 300 mM NaCl. Active fractions were pooled and shown to contain essentially homogeneous Hjc protein. This protein was used for all subsequent analyses.

Site-directed Mutagenesis

Site-directed mutagenesis of Hjc was carried out in the plasmid pUC119 using the QuikChange method (Stratagene). After mutagenesis, DNA sequencing was used to confirm that no spurious mutations had been introduced. The Hjc mutants were subcloned into pET19b and pUC119 using the QuikChange method (Stratagene). After mutagenesis, DNA sequencing was used to confirm that no spurious mutations had been introduced. The Hjc mutants were subcloned into pET19b and pUC119 using the QuikChange method (Stratagene). After mutagenesis, DNA sequencing was used to confirm that no spurious mutations had been introduced. The Hjc mutants were subcloned into pET19b and pUC119 using the QuikChange method (Stratagene). After mutagenesis, DNA sequencing was used to confirm that no spurious mutations had been introduced. The Hjc mutants were subcloned into pET19b and pUC119 using the QuikChange method (Stratagene). After mutagenesis, DNA sequencing was used to confirm that no spurious mutations had been introduced. The Hjc mutants were subcloned into pET19b and pUC119 using the QuikChange method (Stratagene). After mutagenesis, DNA sequencing was used to confirm that no spurious mutations had been introduced. The Hjc mutants were subcloned into pET19b and pUC119 using the QuikChange method (Stratagene). After mutagenesis, DNA sequencing was used to confirm that no spurious mutations had been introduced. The Hjc mutants were subcloned into pET19b and pUC119 using the QuikChange method (Stratagene).

Comparative Gel Electrophoretic Retardation Analysis of Hjc-DNA Junction Interactions

Samples of purified Hjc protein (1 µM) were incubated with radioactive 5'-32P-labeled four-way DNA junction 3 in binding buffer (20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 0.1 mg/ml bovine serum albumin, and 0.2 mg/ml calf thymus duplex competitor DNA) including either 1 mM EDTA or 0.1 mM MgCl2 in a 10-µl total volume for 5 min at 20 °C, prior to addition of loading buffer (0.25% bromphenol blue, 0.25% xylene cyanol FF, 15% Ficoll type 400) at a dilution of 1:6 (v/v). Samples were loaded onto 5% polyacrylamide gels and electrophoresed in Tris-borate-EDTA buffer or Tris-borate and 0.1 mM MgCl2 with buffer recirculation. After electrophoresis, gels were dried on Whatman 3MM paper and exposed to x-ray film for documentation.

Equilibrium Binding of Wild-type and Mutant Hjc Proteins

Binding affinity was measured by gel electrophoretic retardation analysis using radioactive 5'-32P-labeled junction 1 in EDTA binding buffer as described previously (8).

Assay of Hjc Activity

Assays were carried out using 1 µM purified recombinant Hjc protein in reaction buffer (20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 15 mM MgCl2) using 80 nM 5'-32P-labeled junction as a substrate. Calf thymus DNA (0.2 mg/ml) was added as a competitor to minimize nonspecific endonuclease activity and DNA-binding proteins. Reactions were initiated by the addition of magnesium to the assay mix in a 5-µl total volume and incubated at 60 °C. At set time points aliquots were removed, and the reactions were stopped by the addition of 4 µl of formamide/EDTA loading mix with heating to 95 °C. Products were analyzed by denaturing gel electrophoresis and phosphor imaging as described previously (48).

Modification of DNA by Potassium Permanganate

Radioactive 5'-32P-labeled four-way DNA junction Z28 was incubated at room temperature for 5 min in the presence or absence of 1 µM Hjc protein in binding buffer. The total reaction volume was 20 µl. Reactions were initiated by the addition of 2 µl of freshly dissolved 25 mM KMnO4 and were stopped after 1 min with the addition of 1.5 µl of β-mercaptoethanol. After ethanol precipitation, DNA samples were reacted with 100 µl of 1 M piperidine for 30 min at 95 °C. Piperidine was removed by vacuum desiccation, and the pellets were washed three times with 30 µl of water and were vacuum desiccated after each water addition. The dried samples were resuspended in formamide loading mix and analyzed on 15% denaturing polyacrylamide gels.

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**Fig. 1. Comparative gel electrophoretic analysis of the global structure of the four-way junction upon binding of Hjc.** Six species of radioactive 5'-32P-labeled four-way junction 3 were assembled from 16 oligonucleotides such that each species had two long arms of 60 bp and two short arms of 15 bp. Analysis in the absence of magnesium ions. The six junction species were incubated with Hjc in the presence of 1 mM EDTA, and the products were analyzed by gel electrophoresis on a 6% polyacrylamide gel containing 1 mM EDTA. The free junction exhibits the slow-fast-slow-slow-slow pattern characteristic of the extended square configuration of the four-way junction in the absence of metal ions (top left). The complexed species exhibit a fast-intermediate-constant-fast-slow-butterfly pattern, indicating an alteration in the global junction conformation by Hjc. This pattern can be interpreted as a 2-fold symmetric conformation, with the BH and RX pairs related by an acute angle and the BX and HR pairs related by an obtuse angle. B, analysis in the presence of 100 µM magnesium ions. The six junction species were incubated with Hjc in the presence of 100 µM magnesium ions, followed by gel electrophoresis in the presence of 100 µM magnesium ions. The free species migrate in the slow-intermediate-fast-intermediate-slow pattern indicative of the stacked X structure with B upon X coaxial stacking (bottom left). The bound species exhibit the slow-fast-intermediate-constant-fast-slow pattern, as seen in the presence of EDTA, indicating that the bound junction conformation is independent of the presence or absence of magnesium ions. The following junction species were electrophoresed in both A and B. Lane 1, species with long B and H arms; lane 2, species with long B and R arms; lane 3, species with long B and X arms; lane 4, species with long H and R arms; lane 5, species with long H and X arms; lanes 7–12, same as lanes 1–6 with the addition of Hjc protein.
RESULTS

Manipulation of the Global Structure of the Four-way DNA Junction by Hjc—The Holliday junction assumes a conformation known as the “stacked X structure” in the presence of divalent metal ions, folding by coaxial stacking of pairs of helices in an antiparallel 2-fold symmetric cross (reviewed in Ref. 19). This structure has been studied by a wide variety of techniques and has recently been confirmed by X-ray crystallography (20, 21). Comparative gel electrophoresis has proven a powerful technique for the analysis of the global configuration of the four-way junction, both in solution and complexed with proteins. Using this technique, all Holliday junction binding proteins studied to date have been shown to alter the conformation of the Holliday junction on binding (reviewed in Ref. 22).

We examined the effect of Hjc binding on the global conformation of the four-way junction J3 using comparative gel electrophoresis. In EDTA, the free junction species adopted the four-slow/two-fast pattern characteristic of the 4-fold symmetric junction with arms related by 90°. In the protein-DNA complex, this pattern was altered significantly with bound species adopting a distinctive “butterfly” (slow-fast-intermediate-intermediate-slow-fast) pattern (Fig. 1). When the experiment was repeated in the presence of 0.1 mM MgCl₂, the free junction adopted a slow-medium-fast-medium-slow “smile” pattern, as observed previously for J3, which folds with B upon X and H upon R stacking (23). Again, the Hjc-DNA complexes adopted the butterfly pattern. These observations suggest that the four-way DNA junction is bound by Hjc in a 2-fold symmetric conformation with the BX arms and HR arms related by acute angles and the BH and RX pairs related by obtuse angles (Fig. 1). This X shape is reminiscent of that induced on J3 by the resolving enzyme RuvC, although in that case the structure deviated only slightly from 4-fold symmetry and tended toward acute angles between the BH and RX pairs (24). Whereas we cannot quantify the angles between the pairs of arms, the large differences in mobility of the 6 S species suggest that the angles deviate significantly from the 90° observed for Cce1 and RuvA and may approach or surpass a 120°/60° relationship for the obtuse and acute angles.

Using a single turnover kinetic assay, we measured the rates of cleavage of each of the four strands of junction J3 individually. Cleavage was found to be biased, with a 5-fold faster rate observed for the b and r strands compared with the h and x strands (Fig. 2). Relating this to the global structure of J3 in complex with Hjc, the more strongly cleaved strands are situated in the strands exchanging with acute angles, 3 nucleotides 3′ of the branch point (Figs. 1 and 2). Cleavages in the other two strands probably reflect a minor population of the junction that has been bound by Hjc in the other possible conformation with the BX and HR pairs of arms related by acute angles.

Permanganate Probing Suggests Disruption of Base Pairing at the Cleavage Site—Thymine residues in DNA are susceptible to modification by potassium permanganate but are protected from modification in the context of fully stacked duplex DNA. This modification renders the phosphodiester backbone susceptible to cleavage by piperidine. Modification by potassium permanganate is therefore a sensitive probe for base stacking in duplex DNA. Holliday junction binding proteins such as Cce1 and RuvA increase the sensitivity of thymines at the branch point to permanganate modification in the presence of magnesium ions, and this has been interpreted as evidence that the proteins unstack the DNA junction and possibly disrupt base pairing at the point of strand exchange (24, 25).

Permanganate probing was used to analyze base stacking in the context of the Hjc-junction complex with junction Z28, which has eight thymines flanking the point of strand exchange on the r strand. In the absence of Hjc protein, all eight thymines were subject to modification by potassium permanganate, with the strongest reactivity at the 2 nucleotides flank- ing the point of strand exchange and symmetrical distribution with respect to the junction center (Fig. 3A, lane 3). The presence of magnesium in the reaction reduced the sensitivity of the flanking thymines to permanganate, consistent with junction stacking under these conditions (Fig. 3A, lane 6). In the presence of saturating concentrations of the Hjc protein, we observed a marked increase in reactivity with potassium permanganate in both EDTA and magnesium (Fig. 3A, lanes 4 and 7).

Quantification of the increase in reactivity for each of the thymine residues (from positions -4 to +4) revealed a pronounced bias in the reactivity toward the 5′ side of the junction center (Fig. 3B), suggesting the protein unstacks the duplex DNA in this region. Analysis of the cleavage of junction Z28 by Hjc reveals that, similar to junction J3, cleavage is biased, occurring predominantly in the h and x strands 3 nucleotides 3′ of the branch point (Fig. 3C). The position of cleavage of the h strand coincides with the area of enhanced reactivity toward permanganate detected on the r strand, suggesting that Hjc may introduce structural distortion in the DNA helix near the cleavage site. The base pairing in this region of the DNA duplex may be weakened or disrupted by Hjc on binding. Such distortions are common features of DNA recognition by restriction enzymes such as EcoRV (26) and EcoRI (27).

A Conserved Motif in Hjc Resembles the Active Site of Type II Restriction Enzymes—Analysis of the residues conserved in a multiple alignment of all known Hjc sequences revealed a motif containing three acidic residues: Glu-12, Asp-42, and Glu-55 followed by lysine (Lys-57), similar to a conserved motif present in type II restriction enzymes (28) (Fig. 4). This motif constitutes part of the active site of the restriction enzymes, with the
FIG. 3. Base unstacking probed by potassium permanganate. Hjc binding to four-way junction Z28 induces distortion of base stacking that is asymmetrical with respect to the point of strand exchange and correlates with the position of strand cleavage. A, junction Z28, containing four thymine residues on either side of the branch point of strand r, was 5'-32P-labeled on the r strand. The junction was incubated in the presence of 1 mM EDTA or 0.1 mM MgCl₂ with or without 1 μM Hjc protein in binding buffer and was reacted with potassium permanganate as described under “Materials and Methods.” Reactivity to permanganate was observed at all eight thymine positions, and this was enhanced by the addition of Hjc protein. The position of the point of strand exchange is indicated by the white triangles. Lane 1, junction Z28 strand r A+G markers; lane 2, junction Z28 C+T marks; lanes 3 and 4, junction in EDTA reacted with permanganate in the absence and presence of Hjc, respectively; lane 5, control showing junction cleavage background without permanganate modification; lanes 6 and 7, junction in magnesium reacted with permanganate in the absence and presence of Hjc, respectively. B, denaturing gel electrophoretic analysis of the cleavage of each of the four strands of junction Z28 by Hjc. Strong cleavage was observed in the b and x strands, 3 nucleotides 3’ of the point of strand exchange (indicated by arrows in the schematic representation of the junction), with little or no cleavage observed in the b or r strands. The point of cleavage in the h strand opposes the area of increased reactivity to permanganate observed in the r strand. C, the enhancement in reactivity to permanganate was calculated for each of the 8 nucleotide positions in the r strand of junction Z28 by phosphor imaging, and the data from the mean of duplicate experiments were plotted as a histogram. Black bars represent reactivity in EDTA and gray bars reactivity in the presence of magnesium. A bias in the reactivity toward the thymines 5’ of the point of strand exchange is apparent.

FIG. 4. A conserved metal binding domain in Hjc. A, Sulfolobus Hjc sequence from residues 1–60 is shown aligned with that of three type II restriction endonucleases, EcoRV, BglII, and PvuII. Four residues absolutely conserved in all Hjc sequences (Glu-12, Asp-42, Glu-55, and Lys-57) are highlighted in black. These align with residues in all three restriction endonucleases that are known to constitute the binding pocket for the catalytic metal ions at the active site. The secondary structural elements of the three restriction enzymes taken from the crystal structures and the predicted secondary structure of Hjc generated by the program PredictProtein (30) using a multiple alignment of all the Hjc proteins are shown below the respective sequences. H, α-helix (light gray); E, β-sheet (dark gray). There is a good match between the known domain structures of the three restriction enzymes and the predicted structure of the first 60 residues of the Hjc protein. B, cartoon showing common structural elements of EcoRV and Hjc. The positions of the catalytic residues in EcoRV are indicated, with the corresponding numbering for Hjc residues in parentheses.
three acidic residues forming the metal binding pocket for the
two catalytic magnesium ions (29) and the conserved lysine predicted to play a role in stabilizing the transition state during
catalysis (26).

To investigate the predicted secondary structure of Hjc in
this region, we submitted a multiple alignment of seven Hjc proteins to the PredictProtein structure prediction program
(30). The results are outlined in Fig. 4 with the conserved
domain of Hjc aligned with the corresponding domains of
EcoRV, PvuII, and BglII. The predicted secondary structure for
Hjc consists of an α-helix containing the first acidic residue
followed by three β-sheets, in remarkable agreement with the
known secondary structures of the three restriction enzymes
(31).

Roles of Conserved Residues Probed by Site-directed Mutagenesis—To test our model for the metal binding site of the
Hjc protein, we created site-directed mutants of the three con-
served acidic residues (E12Q, D42N, E55Q) and the conserved
lysine (K57A). The mutant proteins were expressed in E. coli
and purified as for the wild-type enzyme. The D42N mutant
was poorly expressed in E. coli, and only small amounts of
protein could be purified. The other three mutants were ex-
pressed at similar levels to the wild-type protein and retained
the ability to bind specifically to the four-way junction J1 with
an affinity comparable with the wild-type Hjc protein (Fig. 5A).
To compare the catalytic activities of the wild-type and mutant
enzymes, we incubated the proteins with the four-way junction
substrate Z28 in cleavage buffer at 60 °C and separated cleav-
age products from uncut substrate by denaturing gel electro-
phoresis (Fig. 5B). A weak residual activity in the mutant
K57A represented a 1000-fold decrease in activity compared
with the wild-type enzyme. Mutants E12Q, D42N, and E55Q
displayed no detectable catalytic activity. All four of these
mutations thus appeared to have had a specific effect on catal-
ysis rather than on substrate binding, consistent with the
functional assignment made by analogy to the equivalent res-
ides in EcoRV and other nuclease.

DISCUSSION

Hjc contains a motif first identified as constituting part of
the active site of the type II restriction enzyme EcoRV. Subse-
quent crystal structures of the restriction enzymes PvuII (32) and
BglII (33) demonstrated that the metal binding pocket is
structurally conserved in an αβ-barrel catalytic domain in the
two enzymes, even though the overall folds are very different.
The type II restriction enzymes EcoRI, BamHI, and Cfr101 also possess this αβ-barrel domain (reviewed in Refs. 34 and 35),
and more recently it has been identified in MutH (35), λ exo-
nuclease (36), and type I and III endonucleases (37). All of these
enzymes have a catalytic (usually N-terminal) domain in the
form of an αβ-barrel with different domains that mediate sub-
unit interactions and DNA recognition (35, 38). The quaternary
structures and functions of these proteins are remarkably di-

ergent. Type II restriction enzymes are dimeric and recognize
and cleave specific nucleotide sequences. MutH is monomeric,
and its activity is regulated by the MutL and MutS proteins
following mismatch DNA recognition (35). However, λ-exonu-
clase, a processive 5'→3' exonuclease that degrades one
strand of a DNA duplex after the formation of a double strand
break, has a toroidal homotrimeric structure (36). Perhaps the
most striking example of the separation of recognition and
catalysis is seen in the restriction enzyme FokI, which has the
catalytic αβ-barrel domain separated from the DNA recogni-
tion domain by a linker (39). Nature has thus repeatedly uti-
lized a catalytic metal ion binding domain efficient in phos-
phodiester bond hydrolysis coupled with an array of specific
DNA and protein recognition elements to solve many diverse
problems in nucleic acid metabolism.

We have shown by site-directed mutagenesis that mutations in
residues Glu-12, Asp-42, Glu-55, and Lys-57 of Hjc all result in
at least a 1000-fold decrease in catalytic activity, though the
mutants retain the ability to bind the four-way junction. By
comparison, mutagenesis of the equivalent residues in EcoRV
results in similar large decreases in catalytic activity without
significantly affecting substrate binding (40–44). This is the
first example of this domain in a Holliday junction resolving
enzyme and the first in an archaeal protein of known function.

Using the sequence alignments shown in Fig. 4, we used the
crystal structure of the metal binding domain of EcoRV to
generate a model of the catalytic domain of Hjc (Fig. 6A). The
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A model encompasses the N-terminal half of the Hjc protein consisting of an α-helix and a three-stranded antiparallel β-sheet. The three acidic residues group in reasonable positions for the catalytic function. Basic residues in Hjc are in reasonable proximity to the negatively charged phosphodiester backbone. Using this constraint, the model suggests a possible mode of binding of the Hjc protein with the four-way DNA junction substrate. Basic residues in Hjc are in reasonable proximity to the negatively charged phosphodiester backbone, and in particular, the model suggests possible roles for two arginines, Arg-13 and Arg-28. These are the only basic residues other than the catalytic Lys-57 that are absolutely conserved in all Hjc sequences, and in the model of the complex they exist in suitable positions to form salt bridges with phosphates on an adjacent arm of the junction. On the basis of the model and the conservation of these residues we predict that they are important for DNA binding and possibly for recognition of the branched structure of the four-way DNA junction.

In conclusion, we have demonstrated that the archaeal Holliday junction resolving enzyme Hjc manipulates the global structure of the four-way DNA junction into a 2-fold symmetric X conformation on binding. Paired nicks in the phosphodiester backbone are introduced on the minor groove face in a region of duplex DNA 3 bp from the point of strand exchange, accompanied by local distortion of base stacking in the duplex. Hjc utilizes an αβ-barrel domain for the chemical step in the resolution of four-way DNA junctions, with four conserved catalytic residues found in many other nucleases of diverse function. The C-terminal half of the Hjc protein may complete the αβ-barrel domain and provide an interface for dimerization. Thus the Hjc dimer may consist of two nuclease domains capable of introducing nicks in duplex DNA on either side of the branch point of a four-way junction, with other structural elements providing structural specificity and forming the dimer interface.

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REFERENCES

1. Iwasaki, H., Takahagi, M., Shiba, T., Nakata, A., and Shinagawa, H. (1991) EMBO J. 10, 4381–4389
2. Connolly, B., Parsons, C. A., Benson, F. E., Dunderdale, H. J., Sharples, G. J., Lloyd, R. G., and West, S. C. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 6063–6067
3. Mahdi, A. A., Sharples, G. J., Mandal, T. N., and Lloyd, R. G. (1996) J. Mol. Biol. 257, 561–573
4. Mizuuchi, K., Kemper, B., Hays, J., and Weisberg, R. A. (1982) Cell 29, 357–365
5. Dickie, P., McFadden, G., and Morgan, A. R. (1987) J. Biol. Chem. 262, 14826–14836
6. de Massey, B., Weisberg, R. A., and Studier, F. W. (1987) J. Mol. Biol. 193, 559–576
7. Kleff, S., Kemper, B., and Sternglanz, R. (1992) EMBO J. 11, 699–704
8. Kvaratskhelia, M., and White, M. F. (2000) J. Mol. Biol. 297, 923–932
9. Komori, K., Sakaue, S., Shinagawa, H., Morikawa, K., and Ishino, Y. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 8873–8878
10. West, S. C., Parsons, C. A., and Pickles, S. M. (1987) J. Biol. Chem. 262, 12752–12758
11. Heathman, S. P., Bryan, R. K., Martin, P. D., Petratos, K., and Wilson, K. S. (1991) EMBO J. 10, 359–376
12. Elborough, K. M., and West, S. C. (1990) EMBO J. 9, 2931–2936
13. Ariyoshi, M., Vassylyev, D. G., Iwasaki, H., Nakamura, H., Shinagawa, H., and Morikawa, K. (1994) Cell 78, 1065–1072
14. Raaijmakers, H., Vix, O., Torlo, I., Golz, S., Kemper, B., and Suck, D. (1999) EMBO J. 18, 1447–1458
15. Saita, A., Iwasaki, H., Ariyoshi, M., Morikawa, K., and Shinagawa, H. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 7470–7474
16. Keeling, P. J., and Doolittle, W. F. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5761–5764
17. Kvaratskhelia, M., and White, M. F. (2000) J. Mol. Biol. 295, 193–202
18. Schefold, M. J., Lilley, D. M. J., and White, M. F. (1998) Biochemistry 37, 7733–7740
19. Lilley, D. M. J. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 9513–9515
20. Lilley, D. M., and Norman, D. G. (1999) Nat. Struct. Biol. 6, 897–899
21. Ortiz-Lombardia, M., Gonzalez, A., Eritja, R., Azorin, F., and Coll, M. (1999) Nat. Struct. Biol. 6, 713–718
22. White, M. F., Giraud-Panis, M.-J. E., Pohler, J. R., and Lilley, D. M. J. (1997) J. Mol. Biol. 269, 647–664
23. Murchie, A. J. H., and Coll, M. (1991) EMBO J. 10, 1447–1456
24. Bennett, R. J., and West, S. C. (1993) J. Mol. Biol. 232, 213–226
25. White, M. F., and Lilley, D. M. J. (1997) J. Mol. Biol. 266, 122–134
26. Winkler, F. K., Banner, D. W., Oefner, C., Tserngouli, D., Brown, R. S., Heathman, S. P., Bryan, R. K., Martin, P. D., Petratos, K., and Wilson, K. S.
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(1993) EMBO J. 12, 1781–1795
27. McClarin, J. A., Frederick, C. A., Wang, B. C., Greene, P., Boyer, H. W., Grahle, J., and Rosenberg, J. M. (1986) Science 234, 1526–1541
28. Anderson, J. E. (1993) Curr. Opin. Struct. Biol. 3, 24–30
29. Kostrewa, D., and Winkler, F. K. (1995) Biochemistry 34, 683–696
30. Rost, B., and Sander, C. (1993) J. Mol. Biol. 232, 584–589
31. Venclovas, C., and Siksnys, V. (1995) Nat. Struct. Biol. 2, 838–841
32. Athanasiadis, A., Vlassi, M., Kotsifaki, D., Tucker, P. A., Wilson, K. S., and Kokkinidis, M. (1994) Nat. Struct. Biol. 1, 469–475
33. Newman, M., Lunnen, K., Wilson, G., Greici, J., Schildkraut, I., and Phillips, S. E. (1998) EMBO J. 17, 5466–5476
34. Kovall, R. A., and Matthews, B. W. (1999) Curr. Opin. Chem. Biol. 3, 578–583
35. Ban, C., and Yang, W. (1998) EMBO J. 17, 1526–1534
36. Kovall, R. A., and Matthews, B. W. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 7893–7897
37. Davies, G. P., Martin, I., Crowshaw, A., Murray, N. E., and Dryden, D. T. (1999) J. Mol. Biol. 290, 565–579
38. Venclovas, C., Timinskas, A., and Siksnys, V. (1994) Proteins 20, 279–282
39. Wah, D. A., Hirsch, J. A., Dorner, L. F., Schildkraut, I., and Aggarwal, A. K. (1997) Nature 388, 97–100
40. Jeltsch, A., Maschke, H., Selent, U., Wenz, C., Kohler, E., Connolly, B. A., Thorougoud, H., and Pingoud, A. (1995) Biochemistry 34, 6239–6246
41. Selent, U., Ruter, T., Kohler, E., Liedtke, M., Thielking, V., Alves, J., Oelgeschlager, T., Wolfs, H., Peters, F., and Pingoud, A. (1995) Biochemistry 34, 4808–4815
42. Vipond, I. B., and Halford, S. E. (1996) Biochemistry 35, 1701–1711
43. Stanford, N. P., Halford, S. E., and Baldwin, G. S. (1999) J. Mol. Biol. 288, 105–116
44. Groll, D. H., Jeltsch, A., Selent, U., and Pingoud, A. (1997) Biochemistry 36, 11389–11401
45. Duckett, D. R., Murchie, A. I. H., Dieckmann, S., von Kitzing, E., Kemper, B., and Lilley, D. M. J. (1988) Cell 55, 79–89
46. Poehler, J. R. G., Giraud-Panis, M.-J. E., and Lilley, D. M. J. (1996) J. Mol. Biol. 260, 678–696
47. White, M. F., and Lilley, D. M. J. (1996) J. Mol. Biol. 257, 330–341
48. White, M. F., and Lilley, D. M. J. (1997) Mol. Cell. Biol. 17, 6465–6471
49. Sali, A., and Blundell, T. L. (1993) J. Mol. Biol. 234, 779–815
50. Brünger, A. T. (1983) in X-PLOR: A System for X-ray Crystallography and NMR, Yale University Press, New Haven, CT