Biochemical characterization and functional complementation of ribonuclease HII and ribonuclease HIII from *Chlamydomonas reinhardtii* AR39

Rubing Liang, Xipeng Liu, Dongli Pei and Jianhua Liu

Correspondence

Jianhua Liu

jianhualiudl@sjtu.edu.cn

College of Life Science and Technology, Shanghai Jiaotong University, 800 Dong-Chuan Road, Shanghai 200240, China

Chlamydomonas reinhardtii AR39 contains two different ORFs (CP0654 and CP0782) encoding ribonuclease H (RNase H) homologues, Cpn-RNase HII and Cpn-RNase HIII. Sequence alignments show that the two homologues both contain the conserved motifs of type 2 RNase H, and Cpn-RNase HII has the conserved active-site motif (DEDD) of RNase HII. Cpn-RNase HIII also contains a unique active-site motif (DEDE), common to other RNase HIIIs. Complementation assays indicated that Cpn-RNase HII can complement both *Escherichia coli* RNase HII and RNase HI, but Cpn-RNase HIII can only complement the latter. *In vitro* enzyme activity experiments showed that neither Cpn-RNase HII nor Cpn-RNase HIII is thermostable and their optimum pH values were 9.0 and 10.0, respectively. Cpn-RNase HII cleaves a 12 bp RNA–DNA substrate at multiple sites, but Cpn-RNase HIII at only one site. When a 35 bp DNA–RNA–DNA/DNA chimeric substrate was used, cleavage was only observed with Cpn-RNase HII. These results indicate that the RNase H combination of *C. reinhardtii* AR39 is not simple substitution of *E. coli* RNase H, perhaps representing a more primordial type. This is believed to be the first *in vivo* functional study of *Chlamydomonas* RNase Hs and the results should contribute to the analysis of RNase Hs of other parasite species.

Received 13 October 2006

Revised 5 December 2006

Accepted 7 December 2006

INTRODUCTION

Ribonuclease H (RNase H) endonucleolytically hydrolyses the RNA strand of RNA–DNA hybrids in the presence of divalent cations; it is involved in DNA replication, repair, and/or transcription processes and widely present in all three kingdoms (Ohtani *et al.*, 1999a). RNase Hs have been classified into type 1 and type 2 RNase H, and prokaryotic RNase Hs are divided into three groups, RNase HI, HII and HIII, encoded by *rnhA*, *rnhB* and *rnhC*, respectively (Ohtani *et al.*, 1999a, b). RNase HI is a type 1 RNase H, while RNase HII and HIII are both of type 2.

Although many bacteria, such as *Escherichia coli*, contain both type 1 and type 2 RNase H, some bacteria, such as *Bacillus stearothermophilus*, *Streptococcus pneumoniae*, *Chlamydia trachomatis* and *Aquifex aeolicus* only have two different type 2 RNase Hs (Ohtani *et al.*, 1999a). Although the crystal structures of some RNase HI and HII enzymes have been determined, e.g. *E. coli* (Katayanagi *et al.*, 1990;

Yang *et al.*, 1990), *Methanococcus jannaschii* (Lai *et al.*, 2000) and *Thermococcus kodakaraensis* (Muroya *et al.*, 2001), little 3D structural information on RNase HIII is available. The enzymic properties of a few RNase HIIIs such as *Bacillus subtilis* RNase HIII and *B. stearothermophilus* RNase HIII have been analysed (Ohtani *et al.*, 1999b; Chon *et al.*, 2004, 2006a). Except for the conserved motifs, all the described type 2 RNase Hs contain unique active-site residues, a DEDD motif (Asp–Glu–Asp–Asp) for RNase HII and a DEDE motif (Asp–Glu–Asp–Glu) for RNase HIII (Chon *et al.*, 2006a).

Chlamydomonas reinhardtii, a pathogenic eubacterial intracellular parasite for humans and some animals, infects the mucosal surfaces of the respiratory tract, causing pharyngitis, bronchitis and pneumonia (Hahn *et al.*, 2002). The complete genome sequence of *C. reinhardtii* AR39 offered much genetic information about this microbe (Read *et al.*, 2000). Two prospective RNase HII and HIII genes, CP0654 and CP0782, have been isolated from *C. reinhardtii* AR39 and expressed in *E. coli* previously (Pei *et al.*, 2005). In this study, the enzymic properties of these two RNase Hs (Cpn-RNase HII and HIII) were analysed further and their functional complementation of RNase H-deficient *E. coli* mutants was demonstrated *in vivo*.

Abbreviations: Cpn-RNase HII and HIII, *Chlamydomonas reinhardtii* AR39 RNase HII and HIII; Bsu-RNase HIII, *Bacillus subtilis* RNase HIII; Bst-RNase HIII, *Bacillus stearothermophilus* RNase HIII; Spn-RNase HIII, *Streptococcus pneumoniae* RNase HIII; Ctr-RNase HIII, *Chlamydia trachomatis* RNase HIII.

METHODS

Strains and reagents. *E. coli* strain DY329 [W3110 Δ lacU169 *nadA*::Tn10 *gal490* λ c1857 Δ (*cro-bioA*); Yu *et al.*, 2000] was used in this study; the plasmids used are listed in Table 1. Bacteria were cultured in Luria–Bertani (LB) medium at 32 °C and antibiotics were used as follows: ampicillin, 100 μ g ml^{−1}, kanamycin, 50 μ g ml^{−1} and tetracycline 10 μ g ml^{−1}. Oligonucleotides were synthesized by Invitrogen. Restriction enzymes and DNA-modifying enzymes were purchased from TaKaRa. All other chemicals were analytical grade.

Generation of RNase H-deficient *E. coli* mutants and complementation

DNA manipulation. *E. coli* genomic DNAs were extracted using a genomic DNA extraction and purification kit (Sangon, Shanghai, China) and plasmids were isolated from *E. coli* by the alkaline method (Birnboim & Doly, 1979). PCR reactions were carried out using *LA-Taq* DNA polymerase (TaKaRa) and products were recovered with a gel purification kit (Sangon). Homologous recombination was carried out as described before (Yu *et al.*, 2000) to perform complementation assays. All constructs were confirmed by DNA sequencing (Invitrogen).

Construction of plasmids. To achieve constitutive expression of the genes under study, the GAPDH-promoter fragment was amplified from genomic DNA with GAPDH-F/GAPDH-R (F: 5'-GGGGGGGGATCCgctcacatctcactttaatcg-3'; R: 5'-GGGGGAAGCT-TGCATGCGAGCTCGTCGACGGTACCTGCAGccaccagctattttagtg-3'; F and R represent forward and reverse primers respectively, the underlined bases are introduced to create restriction sites for DNA cloning and the lower-case bases can match the template, but the capitalized bases are added for DNA cloning or homologous recombination), containing the GAPDH-promoter sequence, RBS and recognition sites for several enzymes (*Xho*I, *Kpn*I, *Sac*I, *Sph*I and *Hind*III). The fragment was treated with *Bam*HI/*Hind*III and ligated with predigested pUC18, producing pUC-G (Table 1).

Amplified *rnhA* and *rnhB* fragments from genomic DNA using primers *rnhA*-F/*rnhA*-R (F: 5'-GGGGGGCTCGAGatgcttaaacaggtagaatttccacc-3'; R: 5'-GGGGGGGCATGCTtaacttcaacttgtagctgtatct-3') and *rnhB*-F/*rnhB*-R (F: 5'-GGGGGGCTCGAGatgctgaattgtttatccgacagagctggttgcgg-3'; R: 5'-GGGGGGGCATGCTcaggacgcaagtcagtgcggtttgaca-3'). The CP0654 and CP0782 fragments were amplified from pET28a-CP0654 and pET28a-CP0782 (Pei *et al.*, 2005; Table 1) with primers CP0654-F/CP0654-R (F: 5'-GGGGGGCTCGAGatgaatacttctatttctgaattc-3'; R: 5'-GGGGGGGCATGCTcatacatagcacacatttgc-3') and CP0782-F/CP0782-R (F: 5'-GGGGGGCTCGAGatgctcctgcatgccgcccacc-3'; R: 5'-GGGGGGGCATGCTtatttcccgaacaaatttcgtca-3'). These fragments were treated with *Xho*I/*Sph*I and ligated with

predigested pUC-G, producing pUC-*rnhA*, pUC-*rnhB*, pUC-CP0654 and pUC-CP0782, respectively (Table 1). Another *rnhB* fragment amplified with primers *rnhB*-F2/*rnhB*-R2 (F: 5'-GGGGGGGCATGCTggtggatgatcgaatttgttatccgacacgcagctggttgcgg-3'; R: 5'-GGGGGA-AGCTTtcaggacgcaagtcagtgcggtttgaca-3') was digested with *Sph*I/*Hind*III and ligated into pUC-*rnhA* to produce pUC-*rnhA* + B (Table 1).

Complementation assay. Plasmids pUC-*rnhA*, pUC-*rnhB*, pUC-*rnhA* + B, pUC-CP0654 or pUC-CP0782 were electro-transformed into DY329 and these strains were named as DY-*rnhA*, DY-*rnhB*, DY-*rnhA* + B, DY-CP0654 and DY-CP0782, respectively. Then electro-competent cells of these strains were made (Yu *et al.*, 2000).

The tetracycline and kanamycin cassettes were amplified using primers Tc-F/Tc-R (F: 5'-TACAGTTCGATTCAATTACAGGAAGTCTACCAAGatgaaatctaacaatgcgctcatcg-3'; R: 5'-GCCTGTGGTTTACGACATTGCCGGGTGGCTCCAACtcaggctcaggtggccc-3') and Km-F/Km-R (F: 5'-TGAGCAGGCGGCACAAGCCGTTCTGGAGTTAGCACAATGAgcatattcaacggga-3'; R: 5'-ACATCTTCAGATTCCGGTTTACTTAATCTCGACACAAGAAgaaaaactcatcgagcatca-3') to replace the chromosomal *rnhA* and *rnhB*. Note that most of *rnhA* and *rnhB*, but not the whole of either gene, was substituted, as the promoters of other essential genes were located at the end of their coding sequences, which was retained in our constructed strains. Then the two cassettes were electro-transformed into DY329, DY-*rnhA*, DY-*rnhB*, DY-*rnhA* + B, DY-CP0654 or DY-CP0782. The transformants were screened with appropriate antibiotics and the plates were photographed with a digital camera (Kodak DX6490).

In vitro enzymic activity assays

Recombinant Cpn-RNase Hs were expressed and purified, and the enzymic activities were measured as before (Pei *et al.*, 2005). Unless specified otherwise, assays were performed in reaction buffer containing 10 mM Tris/HCl, pH 9.0, 50 mM NaCl, 1 mM β -mercaptoethanol, 10 mM MgCl₂ and 10 μ g bovine serum albumin ml^{−1}. Reactions were stopped by adding an equal volume of stopping buffer (100 mM EDTA, 8 M urea, 0.1 % bromophenol blue and 0.1 % xylene cyanol) and analysed by electrophoresis on 20 % polyacrylamide gels (with 8 M urea), followed by measurements with an FLA-5000 phosphorimager.

Kinetic parameters. For determination of kinetic parameters, the enzyme was used at 0.01 μ M and the concentration of the 12 bp RNA–DNA substrate varied from 0.052 to 2 μ M. The reaction was performed at 30 °C for 1 min and stopped by adding stopping buffer. The kinetic parameters were determined from Lineweaver–Burk plots.

Table 1. Plasmids used in this study

Plasmid	Genotype	Source
pET28a-CP0654	CP0654 cloned into MCS of pET28a, Km ^r	Pei <i>et al.</i> (2005)
pET28a-CP0782	CP0782 cloned into MCS of pET28a, Km ^r	Pei <i>et al.</i> (2005)
pUC-G	pUC18 derivative containing GAPDH promoter and RBS, Ap ^r	This study
pUC- <i>rnhA</i>	pUC18 derivative containing GAPDH-promoter-controlled <i>rnhA</i> , Ap ^r	This study
pUC- <i>rnhB</i>	pUC18 derivative containing GAPDH-promoter-controlled <i>rnhB</i> , Ap ^r	This study
pUC- <i>rnhA</i> + B	pUC18 derivative containing GAPDH-promoter-controlled <i>rnhA</i> and <i>rnhB</i> , Ap ^r	This study
pUC-CP0654	pUC18 derivative containing GAPDH-promoter-controlled CP0654, Ap ^r	This study
pUC-CP0782	pUC18 derivative containing GAPDH-promoter-controlled CP0782, Ap ^r	This study

pH effect. pH effects were determined by performing the assay as above except that the reaction buffer was substituted with 10 mM imidazole/HCl (pH 5.0–6.5), 10 mM Tris/HCl (pH 7.0–9.0) or 10 mM glycine/NaOH (pH 9.0–12.0).

Temperature effect. Recombinant Cpn-RNase Hs (2 nM) were dissolved in reaction buffer and treated at 70 °C for different times; the temperature effects were analysed by determining the remaining enzymic activities.

Substrate specificity. A 12 bp RNA–DNA (5′-³²P-cggagaugacgg-3′, 3′-GCCTCTACTGCC-5′; note that ribonucleotides are shown by lower-case letters), 35 bp DNA–RNA–DNA/DNA (5′-³²P-AGAG-GCAGGAGAGTtGCAACGCAGCAAGAGGACGC-3′, 3′-TCTCCG-TCTCTCAACGTTGCGTCGTTCTCTGCG-5′) and Ribo primer (5′-³²P-GCGTCCTCTT GCTGCGTTGCaa-3′, 3′-CGCAGGAGAAC-GACGCAACGTTGAGAGGACGGAGA-5′) were used as substrates. All oligonucleotides carrying ribonucleotide(s) were 5′-end-labelled with ³²P and the hybrid duplexes were prepared by standard procedures (Katayanagi *et al.*, 1990). The cleavage assays were performed in reaction mixture containing 0.2 µM of different ribonucleotide substrates and specified amounts of Cpn-RNase Hs.

RESULTS

Complementation of RNase H-deficient *E. coli* with Cpn-RNase HII (CP0654) and Cpn-RNase HIII (CP0782)

According to the BLAST results, two ORFs (CP0654 and CP0782) are presumed to encode Cpn-RNase HII and Cpn-RNase HIII. Cpn-RNase HII is composed of 214 amino acid residues, and has 41 % identity with *E. coli* RNase HII. Cpn-RNase HIII consists of 301 amino acid residues and the sequence identities are 31 %, 30 %, 28 % and 62 % with the RNase HIIIs from *B. subtilis* (Bsu-RNase HIII), *B. stearothermophilus* (Bst-RNase HIII), *S. pneumoniae* (Spn-RNase HIII) and *C. trachomatis* (Ctr-RNase HIII), respectively. Three motifs of type 2 RNase H (G-X-D-E-X-G-X-G, D-S-K-X-L and V/I-A-A-S-I-I-L-A-K/R, where X represents any amino acid residue) are also conserved in both Cpn-RNase Hs, corresponding to Gly³¹-Gly³⁸, Asp⁶²-Leu⁶⁶ and Val¹⁵⁰-Arg¹⁵⁸ of Cpn-RNase HII, and Gly⁹³-Gly¹⁰⁰, Asp¹²⁹-Leu¹³³ and Val²³²-Arg²⁴⁰ of Cpn-RNase HIII. In addition, four acidic active-site residues are also found conserved. In Cpn-RNase HII, these are Asp³³, Glu³⁴, Asp¹²⁷ and Asp¹⁴⁴, and in Cpn-RNase HIII they are Asp⁹⁵, Glu⁹⁶, Asp¹⁹⁷ and Glu²²⁷ (Fig. 1), consistent with previous reports that the active-site motif is conserved as DEDD in RNase HII, but DEDE in RNase HIII (Chon *et al.*, 2006a).

To test whether Cpn-RNase HII and Cpn-RNase HIII could complement the RNase H-deficient *E. coli*, a complementation assay was performed as described in Methods. As expected, *rnhA* and *rnhB* could rescue the corresponding RNase H-deficient mutants (DY-*rnhA*, DY-*rnhB* and DY-*rnhA* + B; data not shown). Cpn-RNase HII (strain CP0654) could complement both the two single RNase H knockout mutants and the double RNase H-deficient mutant, but Cpn-RNase HIII (strain DY-CP0782) could only rescue the *rnhA* knockout mutant. PCR detection also confirmed these

results (data not shown). These results demonstrated (1) Cpn-RNase HII (CP0654) and Cpn-RNase HIII (CP0782) could both complement the RNase HI-knockout *E. coli*; (2) only Cpn-RNase HII (CP0654) could complement the RNase HII-knockout *E. coli*; and (3) Cpn-RNase HII (CP0654) could rescue the RNase H-deficient *E. coli* mutant.

Effects of pH and temperature on the enzymic activity of Cpn-RNase Hs

To analyse the enzyme properties of Cpn-RNase Hs, their kinetic parameters were first determined. The K_m and V_{max} values of Cpn-RNase HII were 1.6 µM and 0.022 µM min⁻¹, while those of Cpn-RNase HIII were 0.644 µM and 0.0135 µM min⁻¹ (about 2-fold lower and 2.5-fold higher, respectively, than those of Cpn-RNase HIII). Errors were below 10 % of the reported value.

pH experiments showed that both the Cpn-RNase Hs exhibited activities at an alkaline pH, consistent with other RNase Hs (Ohtani *et al.*, 1999b; Chon *et al.*, 2006b). The optimal pH for Cpn-RNase HII was 9.0 and for Cpn-RNase HIII, 10.0. The enzymic activities decreased rapidly at pH values below 7.0; activities also decreased at pH values above 10.0, but slowly (Fig. 2).

After incubating for 8 min at 70 °C, only 50 % activity of the two Cpn-RNase Hs remained. And 30 min later, no activity could be detected (Fig. 3). Thus neither Cpn-RNase H was a heat-stable enzyme, consistent with a previous report that the thermostability of RNase Hs often correlates with the growth temperature of the organism (Chon *et al.*, 2004).

Substrate specificities of Cpn-RNase Hs

Three oligonucleotide substrates were used to analyse the substrate specificities of Cpn-RNase Hs. For the 12 bp RNA–DNA duplex, Cpn-RNase HII cleaved it at multiple sites, preferentially at c10-g11, slightly at u7-g8, and very slightly at u7-g11 (Fig. 4a). As to Cpn-RNase HIII, only a6-u7 was the favoured cleavage site (Fig. 4b); this behaviour was quite different from that of other RNase HIIIs such as Bsu-RNase HIII (Ohtani *et al.*, 1999b) and Bst-RNase HIII (Chon *et al.*, 2004), both with multiple-site cleavages. When the 35 bp DNA–RNA–DNA/DNA was used as substrate, only Cpn-RNase HII could cleave it (Fig. 5). And as to the Ribo primer (one primer with two ribonucleotides at the 3′-end), no cleavage was observed with any Cpn-RNase H (data not shown). These results indicate that both the Cpn-RNase Hs could cleave RNA–DNA duplex, but could not use Ribo primer as target, and only Cpn-RNase HII could cleave a hybrid with RNA flanked by DNA.

DISCUSSION

In this study, we analysed the characteristics of two *C. pneumoniae* RNase Hs by sequence alignments, complementation assay and biochemical experiments. Because no RNase HIII homologues existed in *E. coli* and no effective

Fig. 1. Sequence alignment results. Amino acid sequences of Bst-RNase HIII (*B. stearothermophilus*, Bst), Bsu-RNase HIII (*B. subtilis*, Bsu), Spn-RNase HIII (*S. pneumoniae*, Spn), Ctr-RNase HIII (*C. trachomatis*, Ctr), Cpn-RNase HIII (*C. pneumoniae*, CpnIII), and Cpn-RNase HII (*C. pneumoniae*, CpnII) are shown (accession no. AB179782 for Bst; Z75208 for Bsu; U93576 for Spn; AE001273 for Ctr; AAF38581 for CpnIII; and AAF73691 for CpnII). The numbers represent residue positions relative to the initiator methionine for each protein and gaps are denoted by dashes. The residues conserved in at least three different proteins are highlighted in grey, and four active-site residues are indicated with arrows. The motifs conserved in type 2 RNase H are shown as broken lines below the alignment; the secondary structure of Bst-RNase HIII is shown above the sequences.

complementation system could be used in other organisms containing RNase HIII, the complementation assay was performed with *E. coli* RNase HI and HII mutants. The results indicated that Cpn-RNase HII (CP0654) and Cpn-RNase HIII (CP0782) both belong to the type 2 RNase H group, which could complement *E. coli* RNase H mutants *in vivo* and exhibit RNase H activity *in vitro*. This is consistent with previous reports that type 2 RNase H could complement RNase HI-deficient *E. coli*, such as the RNase

HII/III from *B. subtilis* (Ohtani *et al.*, 1999a) or *B. stearothermophilus* (Chon *et al.*, 2004). Further different properties of the two proteins imply that Cpn-RNase HII may be the major RNase H of *C. pneumoniae* and the RNase HII/HIII combination in *C. pneumoniae* AR39 is not a simple substitution of *E. coli* RNase HI/II.

Although many organisms contain two types of RNase H genes in one cell, such as *E. coli* (AB type), multiple type 2

Fig. 2. pH effect on Cpn-RNase Hs. The assay was performed in reaction buffer with pH value varied from 5.0 to 12.0; the initial rates of Cpn-RNase HII (●) and HIII (○) activity are plotted.

RNAse H genes (BC type) have been revealed in some organisms, e.g. *B. subtilis* (Ohtani *et al.*, 1999a) and *B. stearothermophilus* (Chon *et al.*, 2004). Therefore, the presence of two type 2 RNase Hs in *C. pneumoniae* AR39 may not be unusual. Further genome database analysis also showed that the two type 2 RNase H homologues are conserved in other *Chlamydomonas* and *Chlamydia* species, such as *Chlamydomonas abortus*, *Chlamydia muridarum* Nigg, *Chlamydomonas felis* Fe/C-56 and *Chlamydomonas caviae* GPIC, with very high identities with the Cpn-RNase Hs. But up to now, except for the RNase Hs of *C. pneumoniae* AR39, the enzymic properties and biological function of other *Chlamydomonas* RNase Hs have not been determined. Interestingly, *A. aeolicus*, a hyperthermophilic hydrogen-oxidizing bacterium similar to primordial forms of life (Deckert *et al.*, 1998), also only contains two type 2 RNase H homologues. Considering the evolutionary position of these organisms, the RNase H genes of *A. aeolicus* and *Chlamydomonas* perhaps represent an ancestral

Fig. 3. Temperature effect on Cpn-RNase Hs. The Cpn-RNase Hs were pre-incubated at 70 °C for 0–30 min before the activity assay. The initial rates of heat-treated Cpn-RNase HII (●) and HIII (○) activity are plotted.

Fig. 4. Cleavage of the 12 bp RNA–DNA substrate by Cpn-RNase HII (a) and Cpn-RNase HIII (b). Only the ribonucleotides are shown. Cleavage sites are marked on the left with arrows; different sizes of arrows reflect the relative cleavage intensities. Lane 1, a 12 base hydrolysis ladder prepared by incubation at 90 °C for 5 min in 100 mM Na₂CO₃ (pH 9.0); lanes 2–5, hydrolysate with 50 mM Mg²⁺ (lane 2), 10 mM Mg²⁺ (lane 3), 1 mM Mg²⁺ (lane 4) or 0.1 mM Mg²⁺ (lane 5); lane 6, untreated substrate.

Fig. 5. Cleavage of 35 bp RNA–DNA substrate by Cpn-RNase HII and HIII. (a) Lane 1, untreated substrate; lane 2, cleavage of Cpn-RNase HII; lane 3, cleavage of Cpn-RNase HIII. (b) The cleavage site of substrate by Cpn-RNase HII is shown with arrows; the lower-case letter represents the ribonucleotide.

structure and the BC type is perhaps more primordial than the AB type.

The main function of RNase H is to remove RNA primers from Okazaki fragments, process R loops to modulate replication initiation and restore DNA topology (Broccoli *et al.*, 2004; Kogoma & Foster 1998; Arudchandran *et al.*, 2000). Recently, a mitochondrial RNase H from the parasitic protozoon *Leishmania* was analysed, which is essential for the parasite's survival (Misra *et al.*, 2005). This may give a clue to the biological status of the Cpn-RNase Hs, which may play an important role on the DNA replication/translation processes of *Chlamydomonas* and may be vital for this organism. As to the unique biochemical function of RNase Hs, any differential biochemistry and molecular biology of these enzymes in a parasite as compared to that of its host could be exploited for rational drug design against the parasite infection. Therefore, the characterization of Cpn-RNase Hs will be an important avenue toward the development of anti-*Chlamydomonas* compounds.

Moreover, RNase Hs is a member of the 'polynucleotide transferases' superfamily, including resolvase (Ariyoshi *et al.*, 1994), integrase (Maignan *et al.*, 1998), transposase (Rice & Mizuuchi, 1995), exonuclease III (Mol *et al.*, 1995), type II restriction enzymes (Kostrewa & Winkler, 1995), Vsr endonuclease (Tsutakawa *et al.*, 1999), RNA helicase (Nishino *et al.*, 2003) and RNase III (Błaszczuk *et al.*, 2001). These enzymes have homologous active sites and are likely to share a common mechanism for catalysis. Hence, understanding of RNase H may contribute to the study of other members.

ACKNOWLEDGEMENTS

This work was partially supported by the National Basic Research Program of China (Grant no. 2005CB724301) and the National Science Foundation of China (Grant no. 30571012/C011003).

REFERENCES

- Ariyoshi, M., Vassilyev, D. G., Iwasaki, H., Nakamura, H., Shinagawa, H. & Morikawa, K. (1994). Atomic structure of the RuvC resolvase: a Holliday junction-specific endonuclease from *E. coli*. *Cell* **78**, 1063–1072.
- Arudchandran, A., Cerritelli, S., Narimatsu, S., Itaya, M., Shin, D. Y., Shimada, Y. & Crouch, R. J. (2000). The absence of ribonuclease H1 or H2 alters the sensitivity of *Saccharomyces cerevisiae* to hydroxyurea, caffeine and ethyl methanesulphonate: implications for roles of RNases H in DNA replication and repair. *Genes Cells* **5**, 789–802.
- Birnboim, H. C. & Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res* **7**, 1513–1523.
- Błaszczuk, J., Tropea, J. E., Bubunencko, M., Routzahn, K. M., Waugh, D. S., Court, D. L. & Ji, X. (2001). Crystallographic and modeling studies of RNase III suggest a mechanism for double-stranded RNA cleavage. *Structure* **9**, 1225–1236.
- Broccoli, S., Rallu, F., Sanscartier, P., Cerritelli, S. M., Crouch, R. T. & Drolet, M. (2004). Effects of RNA polymerase modifications on transcription-induced negative supercoiling and associated R-loop formation. *Mol Microbiol* **52**, 1769–1779.
- Chon, H., Nakano, R., Ohtani, N., Haruki, M., Takano, K., Morikawa, M. & Kanaya, S. (2004). Gene cloning and biochemical characterizations of a thermostable ribonuclease HIII from *Bacillus stearothermophilus*. *Biosci Biotechnol Biochem* **68**, 2138–2147.
- Chon, H., Matsumura, H., Koga, Y., Takano, K. & Kanaya, S. (2006a). Crystal structure and structure-based mutational analyses of RNase HIII from *Bacillus stearothermophilus*: a new type 2 RNase H with TBP-like substrate-binding domain at the N terminus. *J Mol Biol* **356**, 165–178.
- Chon, H., Tadokoro, T., Ohtani, N., Koga, Y., Takano, K. & Kanaya, S. (2006b). Identification of RNase HII from psychrotrophic bacterium, *Shewanella* sp. SIB1 as a high-activity type RNase H. *FEBS J* **273**, 2264–2275.
- Deckert, G., Warren, P. V., Gaasterland, T., Young, W. G., Lenox, A. L., Graham, D. E., Overbeek, R., Snead, M. A., Keller, M. & other authors (1998). The complete genome of the hyperthermophilic bacterium *Aquifex aeolicus*. *Nature* **392**, 353–358.
- Hahn, D. L., Azenabor, A. A., Beatty, W. L. & Burne, G. I. (2002). *Chlamydia pneumoniae* as a respiratory pathogen. *Front Biosci* **7**, e66–e76.
- Katayanagi, K., Miyagawa, M., Matsushima, M., Ishikawa, M., Kanaya, S., Ikehara, M., Matsuzaki, T. & Morikawa, K. (1990). Three-dimensional structure of ribonuclease H from *E. coli*. *Nature* **347**, 306–309.
- Kogoma, T. & Foster, P. L. (1998). Physiological functions of *E. coli* RNase HI. In *Ribonucleases H*, pp. 39–66. Edited by R. J. Crouch & J. J. Toulme. Paris: INSERM.
- Kostrewa, D. & Winkler, F. K. (1995). Mg²⁺ binding to the active site of EcoRV endonuclease: a crystallographic study of complexes with substrate and product DNA at 2 Å resolution. *Biochemistry* **34**, 683–696.
- Lai, L., Yokota, H., Hung, L. W., Kim, R. & Kim, S. H. (2000). Crystal structure of archaeal RNase HII: a homologue of human major RNase H. *Structure* **8**, 897–904.
- Maignan, S., Guilloteau, J. P., Zhou-Liu, Q., Clement-Mella, C. & Mikol, V. (1998). Crystal structures of the catalytic domain of HIV-1 integrase free and complexed with its metal cofactor: high level of similarity of the active site with other viral integrases. *J Mol Biol* **282**, 359–368.
- Misra, S., Bennett, J., Friew, Y. N., Abdulghani, J., Irvin-Wilson, C. V., Tripathi, M. K., Williams, S., Chaudhuri, M. & Chaudhuri, G. (2005). A type II ribonuclease H from *Leishmania* mitochondria: an enzyme essential for the growth of the parasite. *Mol Biochem Parasitol* **143**, 135–145.
- Mol, C. D., Kuo, C. F., Thayer, M. M., Cunningham, R. P. & Tainer, J. A. (1995). Structure and function of the multifunctional DNA-repair enzyme exonuclease III. *Nature* **374**, 381–386.
- Muroya, A., Tsuchiya, D., Ishikawa, M., Haruki, M., Morikawa, M., Kanaya, S. & Morikawa, K. (2001). Catalytic center of an archaeal type 2 ribonuclease H as revealed by X-ray crystallographic and mutational analyses. *Protein Sci* **10**, 707–714.
- Nishino, T., Komori, K., Ishino, Y. & Morikawa, K. (2003). X-ray and biochemical anatomy of an archaeal XPF/Rad1/Mus81 family nuclease: similarity between its endonuclease domain and restriction enzymes. *Structure* **11**, 445–457.
- Ohtani, N., Haruki, M., Morikawa, M. & Kanaya, S. (1999a). Molecular diversities of RNase H. *J Biosci Bioeng* **88**, 12–19.
- Ohtani, N., Haruki, M., Morikawa, M., Crouch, R. J., Itaya, M. & Kanaya, S. (1999b). Identification of the genes encoding Mn²⁺-dependent RNase HII and Mg²⁺-dependent RNase HIII from

Bacillus subtilis: classification of RNases H into three families. *Biochemistry* **38**, 605–618.

Pei, D., Liu, J., Liu, X. & Li, S. (2005). Expression of both *Chlamydia pneumoniae* RNase HIIIs in *Escherichia coli*. *Protein Expr Purif* **40**, 101–106.

Read, D., Brunham, R. C., Shen, C., Gill, S. R., Heidelberg, J. F., White, O., Hickey, E. K., Peterson, J., Utterback, T. & other authors (2000). Genome sequences of *Chlamydia trachomatis* MoPn and *Chlamydia pneumoniae* AR 39. *Nucleic Acids Res* **28**, 1397–1406.

Rice, P. & Mizuuchi, K. (1995). Structure of the bacteriophage Mu transposase core: a common structural motif for DNA transposition and retroviral integration. *Cell* **82**, 209–220.

Tsutakawa, S. E., Jingami, H. & Morikawa, K. (1999). Recognition of a TG mismatch: the crystal structure of very short patch repair endonuclease in complex with a DNA duplex. *Cell* **99**, 615–623.

Yang, W., Hendrickson, W. A., Crouch, R. J. & Satow, Y. (1990). Structure of ribonuclease H phased at 2 Å resolution by MAD analysis of the selenomethionyl protein. *Science* **249**, 1398–1405.

Yu, D., Ellis, H. M., Lee, E. C., Jenkins, N. A., Copeland, N. G. & Court, D. L. (2000). An efficient recombination system for chromosome engineering in *Escherichia coli*. *Proc Natl Acad Sci U S A* **97**, 5978–5983.

Edited by: T. P. Hatch