Unlike the E. coli counterpart, archaeal RNase HII cannot process ribose monophosphate abasic sites and oxidized ribonucleotides embedded in DNA

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Abstract :

The presence of ribonucleoside monophosphates (rNMPs) in nuclear DNA decreases genome stability. To ensure survival despite rNMP insertions, cells have evolved a complex network of DNA repair mechanisms, in which the ribonucleotide excision repair pathway, initiated by type 2 ribonuclease H (RNase HII/2), plays a major role. We recently demonstrated that eukaryotic RNase H2 cannot repair damaged, that is, ribose monophosphate abasic (both apurinic or apyrimidinic) site (rAP) or oxidized rNMP embedded in DNA. Currently, it remains unclear why RNase H2 is unable to repair these modified nucleic acids having either only a sugar moiety or an oxidized base. Here, we compared the endoribonuclease specificity of the RNase HII enzymes from the archaeon Pyrococcus abyssi and the bacterium Escherichia coli, examining their ability to process damaged rNMPs embedded in DNA in vitro. We found that E. coli RNase HII cleaves both rAP and oxidized rNMP sites. In contrast, like the eukaryotic RNase H2, P. abyssi RNase HII did not display any rAP or oxidized rNMP incision activities, even though it recognized them. Notably, the archaeal enzyme was also inactive on a mismatched rNMP, whereas the E. coli enzyme displayed strong preference for the mispaired rNMP over the paired rNMP in DNA. On the basis of our biochemical findings and also structural modeling analyses of RNase HII/2 proteins from organisms belonging to all three domains of life, we propose that RNases HII/2’s dual roles in RER and RNA/DNA hydrolysis result in limited acceptance of modified rNMPs embedded in DNA.

Keywords : type 2 RNase H, Escherichia coli, Bacteria, Pyrococcus abyssi, Archaea, ribose monophosphate abasic (both apurinic or apyrimidinic) site (rAP), oxidized ribonucleotides (r8oxoG), DNA repair, genome integrity, ribonucleoside monophosphate (rNMP)
Unlike the *E. coli* counterpart, archaeal RNase HII cannot process ribose monophosphate abasic sites and oxidized ribonucleotides embedded in DNA.

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Running title: Inability of RNase HII to process modified ribonucleotides

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Keywords: type 2 RNase H, *Escherichia coli*, *Bacteria*, *Pyrococcus abyssi*, *Archaea*, ribose monophosphate abasic (both apurinic or apyrimidinic) site (rAP), oxidized ribonucleotides (r8oxoG), DNA repair, genome integrity, ribonucleoside monophosphate (rNMP)

ABSTRACT

The presence of ribonucleoside monophosphates (rNMPs) in nuclear DNA decreases genome stability. To ensure survival despite rNMP insertions, cells have evolved a complex network of DNA repair mechanisms, in which the ribonucleotide excision repair pathway, initiated by type 2 ribonuclease H (RNase HII/2), plays a major role. We recently demonstrated that eukaryotic RNase H2 cannot repair damaged, that is, ribose monophosphate abasic (both apurinic or apyrimidinic) site (rAP) or oxidized rNMPs embedded in DNA. Currently, it remains unclear why RNase H2 is unable to repair these modified nucleic acids having either only a sugar moiety or an oxidized base. Here, we compared the endoribonuclease specificity of the RNase HII enzymes from the archaean *Pyrococcus abyssi* and the bacterium *Escherichia coli*, examining their ability to process damaged rNMPs embedded in DNA in vitro. We found that *E. coli* RNase HII cleaves both rAP and oxidized rNMP sites. In contrast, like the eukaryotic RNase H2, *P. abyssi* RNase HII did not display any rAP or oxidized rNMP incision activities, even though it recognized them. Notably, the archaeal enzyme was also inactive on a mismatched rNMP, whereas the *E. coli* enzyme displayed strong preference for the mispaired rNMP over the paired rNMP in DNA. On the basis of our biochemical findings and also structural modeling analyses of RNase HII/2 proteins from organisms belonging to all three domains of life, we propose that RNases HII/2’s dual roles in RER and RNA/DNA hydrolysis result in limited acceptance of modified rNMPs embedded in DNA.

A recently recognized type of abundant DNA damage is represented by the incorporation of ribonucleoside monophosphates (rNMPs) into genomic DNA (1–7). rNMPs in DNA can impact genome stability in multiple ways (1). Although a helpful effect was hypothesized, in which nicking by RNase H2 at rNMP sites works as a DNA damage signaling event (8, 9), the most established hypothesis considers the presence of rNMPs within the double helical DNA as harmful for the cells (1, 4, 5, 8). Indeed, it has been demonstrated that the additional hydroxyl group in the 2’ position of the ribose sugar alters and destabilizes the double helix of DNA (10, 11, 4), and its persistent presence can block physiological processes including DNA replication and transcription (1, 12). Several studies have been performed in order to investigate
a putative role of known DNA repair pathways, including nucleotide excision repair (NER) (13) and mismatch repair (MMR) (9, 14), in the removal of rNMPs incorporated in DNA (15). However, to date, only a specific DNA repair pathway, called ribonucleotide excision repair (RER) (16), was shown to have a predominant role in processing single rNMP or short rNMP stretches embedded in DNA. In the RER mechanism, type 2 RNase H proteins cleave the phosphodiester bond at the 5’ side of embedded rNMPs (17, 16). Through a metal – ion dependent cleavage, the RNase H2/II processing generates a product bearing a 5’-phosphate and a 3’-OH end that is subsequently repaired by other enzymes, including DNA polymerase, nuclease and DNA ligase (17, 16). In addition to type 2, RNase H family includes another group called type 1 (18, 14, 7). In mammals, RNase H1 displays incision activity on rNMPs embedded in DNA in a stretch of at least four in series (18). In non-physiological conditions, in which RER pathway components fail or are genetically inactive, RNase H1 cannot substitute RNase H2 for processing single rNMPs in DNA or tracts shorter than four rNMPs. On the other hand, it was clearly demonstrated how Topoisomerase I is able to recognize and cleave single rNMPs embedded in DNA, when RNase H2 is missing (15, 19). Moreover, different studies were carried out to evaluate if base excision repair (BER) pathway could also work on rNMPs embedded in DNA (9, 12, 15, 20). Our laboratories have recently demonstrated that, though BER does not have any role in repairing rNMPs embedded in DNA, apurinic/apyrimidinic endonuclease 1, APE1, the only AP-endonuclease of BER, acts in the removal of modified rNMPs embedded in DNA, such as ribose monophosphate abasic (both apurinic or apyrimidinic) site (rAP) and oxidized rNMP (20). Moreover, on the contrary to what was expected, rAP and oxidized rNMP sites are not processed by yeast, mouse and human RNase H2. Considering all these points, using in vitro assays, we evaluated the activity of RNase HII/2 derived from a representative species from all the three domains of life on different rNMP-containing substrates. Although RNase HII/2 is a phylogenetically conserved enzyme, several differences emerge among RNases HII/2 from different organisms during evolution. Structural studies by X-ray crystallography characterized the different structures among bacterial, archaeal and eukaryotic RNase HII/2 proteins. In both Bacteria and Archaea, RNase HII is a monomeric protein comprising a catalytic core and a small C-terminal helical domain (18). In the hyperthermophilic deep-sea euryarchaeon Pyrococcus abyssi, a single gene rnh is present coding for a single enzyme involved in RNA elimination called type 2 RNase H (Pab RNase HII) (22, 23). Pab RNase HII works in the resolution of RNA primers at the replication fork and in the repair of single rNMPs embedded in DNA (22, 23). Finally, in Eukarya, RNase H2 is a heterotrimeric protein. Subunit A resembles the bacterial orthologue and comprises the catalytic domain of the whole protein. Moreover, two additional subunits, called B and C, are present (24). Although their functions are still not completely understood, their absence abolishes the catalytic activity of the A subunit (17). In addition, subunit B contains the PCNA-interacting motif (PIP-box) that allows recruitment of the enzyme to the replication fork (25). Whether and how the protein structure impacts the protein functions needs further investigation. In this study, we compared the endoribonuclease activities of RNases HII from a bacterial and an archaeon representative species on rAP or oxidized rNMP embedded in DNA. We found that E. coli RNase HII, which is able to cut normal rGMPs embedded in DNA, maintains its endoribonuclease activity on both abasic and oxidized rGMPs present in DNA. On the contrary, Pyrococcus abyssi RNase HII loses this catalytic specificity. Like the human counterpart, the archaeal RNase HII enzyme exhibits stable substrate binding, although it is unable to process modified rGMPs embedded in DNA. Based on these biochemical data, and alignment of amino acid sequences and structures, we speculated that the requirement of the enzymes to recognize rNMPs in DNA and resolve RNA/DNA hybrids limits the optimally recognition process and catalysis of modified rNMPs embedded in DNA.

Results

**Bacterial RNase HII cleaves rAP and oxidized rNMP embedded in dsDNA**

RNase H type 2 is the major enzyme responsible for the removal of unmodified rNMPs embedded in DNA (24); unexpectedly, we recently demonstrated that yeast, mouse and human RNase H2 enzymes are unable to process rAP or oxidized rNMP embedded in DNA (20). Here, we examined whether RNase HII from E. coli cleaves a rAP embedded in a 40-bp DNA sequence (Figure 1A). We used single-stranded (ss) or double-stranded (ds) DNA substrates containing either an rGMP (rG) or a tetrahydrofuran (rF), analog of the rAP (Figure 1A and Table 1). Following incubation,
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denaturing gel electrophoresis was used to visualize the cleavage (Figure 1B). As expected, *E. coli* RNase HII cleaves the single rG in the DNA duplex substrate (lane 9 in Figure 1B). In addition, *E. coli* RNase HII also cleaves rG when present in the ssDNA substrate, although with less efficiency than the dsDNA substrate (lanes 7-8 and 9 in Figure 1B). Interestingly, *E. coli* RNase HII cleaves also the rAP in the dsDNA but not in the ssDNA substrate (lanes 12 and 10-11 in Figure 1B). These results demonstrate that *E. coli* RNase HII is able to cleave a rAP when this is embedded in DNA, as observed by the presence of cleavage products accumulating with ds_rOH:dC (see results in Figure 1). With the purpose to verify that the observed activity of the *E. coli* RNase HII was not a consequence of contamination of the commercial enzyme by RNase A, despite having information about the purity of the protein (~99%), we performed additional control experiments. First, we tested the efficiency of RNase A cleavage on genomic RNA (gRNA) extracted from HeLa cells in comparison with the activity detectable in the presence of increasing amounts of *E. coli* RNase HII (Figure 2C). After 1 hour of RNase A incubation with eukaryotic total RNA, in a ratio [RNase A/gRNA] of 2.5*10^-4, a complete degradation of RNA was observed. Indeed, the two bands corresponding to the 28S and 18S ribosomal RNAs (rRNAs) were sharply visible when RNase A was not present, whereas a complete rRNA degradation was clearly observed when RNase A was present. On the contrary, *E. coli* RNase HII treatment did not affect RNA stability at all (Figure 2C), confirming the absence of RNase A contamination in the *E. coli* RNase HII samples. Using the same conditions, we compared the cleavage activity, by both RNases, on all the rNMP- containing DNA substrates. As shown in Figure 2D, while *E. coli* RNase HII cleaved the substrates containing the modified rNMPs, RNase A did not. These data clearly demonstrate the absence of contaminating RNase A having RNase H-like activity in the commercial RNase HII sample, and, more importantly, that the bacterial RNase HII enzyme, but not RNase A, specifically processes lesions including oxidized rNMPs and rAP embedded in DNA. It is well known that the presence of magnesium ion is necessary for promoting the cleavage activity of *E. coli* RNase HII (20, 26). Here, we investigated the cleavage specificity of the bacterial RNase HII comparing its activity in the presence and absence of magnesium in the reaction buffer. While in the previous experiments the commercial buffer was used, we prepared a home-made buffer, in which we added or not MgSO₄ from the original recipe. As reported in Figure 2E and 2F, when magnesium is present, the activity of RNase HII is promoted in both
Archaeal RNase HII, though being able to recognize both rAP and oxidized rNMP embedded in dsDNA, is unable to process them

In our recent study (20), we demonstrated that yeast, mouse and human RNase H2, although being able to cleave at a single rGMP embedded in DNA, are not capable of cleaving modified rGMPs including oxidized and abasic dsODNs. On the contrary, the type 2 RNase H E. coli counterpart is active on these same substrates. We thought that a loss of this processing activity in eukaryotes could have been fixed during evolution. For this reason, we were intrigued to examine the activity of another type 2 RNase H enzyme present in Archaea, specifically from the P. abyssi (Pab) organism. As described in the Materials and Methods section, the recombinant Pab RNase HII monomeric protein was expressed and purified to homogeneity and carefully quantified (Figure 3A). Considering that this protein exerts its activity at 60 °C in a pH condition around 8.0 (22), we incubated the Pab protein with each rNMP-containing DNA substrate and stopped the reaction at different time-points. As shown in Figure 3B and 3C, the Pab RNase HII protein was highly active on the ds_rG:dC, whereas it was completely inactive on oxidized and abasic dsODNs, ds_r8oxoG:dC and ds_rOH:dC. Markedly, and unlike the E. coli RNase HII (Figure 2), the Pab RNase HII protein was also unable to cleave the rG-containing dsODN, when this was mismatched with adenine in place of cytosine (Figure 3B and 3C). Since the optimal working temperature of the archaeal RNase HII is around 60 °C, close to the temperature of melting (Tm) of the ODNs used in this study (Table 1), we hypothesized that the absence of cleavage activity was due to a possible denaturation of the dsODNs that could hamper the interpretation of the results obtained. To exclude this possibility, we performed a temperature-dependent experiment in order to monitor the processing activity of the Pab RNase HII enzyme when the temperature of reaction was lower than that corresponding to the Tm of the dsODNs (Figure 3D and 3E). Under optimal conditions for the protein (60 °C), Pab RNase HII efficiently cleaved the control substrate ds_rG:dC. The product formation decreased when the temperature was lower than 60°C in a linear manner. These data show that the Pab RNase HII enzyme works more slowly at temperatures lower than its optimal temperature. The activity of Pab RNase HII on the dsODNs containing modified rNMPs was undetectable at all the temperatures tested. These results show that the optimal high temperature of Pab RNase HII is not solely responsible for its inability to process oxidized rNP or rAP sites, as a consequence of a putative non-optimal dsDNA structural conformation. Our results demonstrate that archaeal RNase HII from P. abyssi is not able to process rAP or oxidized rNMP embedded in DNA, like the eukaryotic RNase H2 (20). We then investigated whether the lack of processing activity of Pab RNase HII on rAP and on oxidized rNMP embedded in DNA was due to an inability of binding these substrates. In a recent study, we showed that human RNase H2 is unable to process an rAP and also an oxidized rNMP in DNA (20). Here, we measured the ability of archaeal RNase HII and human RNase H2 to bind the abasic, ds_rOH:dC, and the oxidized, ds_r8oxoG:dC dsODNs, as well as the positive control ds_rG:dC through cross-linking experiments (Figure 3F). As shown in the representative gel, the crosslinking analysis allowed us to detect the formation of a stable protein-DNA complex migrating at around 40 kDa and 55 kDa for Pab RNase HII (lanes 2-5-8) and human RNase H2 (lanes 3-6-9), respectively, with all the substrates used in this study. In light of these results, we conclude that, although the eukaryotic and archaeal RNase HII/2 proteins have no capability to process rAP and oxidized rNMP embedded in DNA, these enzymes maintain the ability to bind them generating a stable DNA-protein complex.

Structural modeling and alignment analyses highlight subtle structural differences in RNase H type 2 enzymes that may affect the spatial positioning of essential amino acids for cleavage

To provide a molecular explanation for the functional differences observed for bacterial, archaeal and eukaryotic RNase HII/2 proteins, we performed an in-silico structural analysis of the bacterial Thermotoga maritima (Tma), Thermus thermophilus (Tth), Escherichia coli (Eco), Saccharomyces cerevisiae (Sac), Homo sapiens proteins, and considered the results of the structural alignment analyses of the type 2 RNase H enzymes.
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(Hsa), Mus Musculus (Mmu), Archaeoglobus fulgidus (Afu), Pyrococcus abyssi (Pab) and Thermococcus kodakarensis (Tko). The alignment was generated using three representative proteins of RNases HII/2 from each kingdom of life, whose sequence diversity was chosen to best illustrate the sequence variability. Moreover, structural modeling is based on the availability of the three-dimensional structures of one representative RNase HII/2 (TmaRNaseHII, HsaRNaseH2 and TkoRNaseHII) from each domain of life. Comparison of secondary structure elements of RNases HII/2 proteins points out conserved residues involved in catalysis, consisting of four highly conserved carboxylates (DEDD motif), the GRG 2′-OH sensing motif, and residues involved in substrate binding (Figure 4A) (27–30).

Interestingly, archaeal and eukaryotic RNases HII/2 contain 2 α-helical structures (namely α1 and α2 according to the secondary structure of Tko RNase HII), which are absent in the bacterial orthologue. Additionally, a short one-turn helix flanked by two other helices is unique to bacterial RNase HII (namely α7 according to the secondary structure of Tma RNase HII). Likewise, the three-dimensional structure of RNases HII/2 in Archaea (Tko RNase HII), Bacteria (Tma RNase HII) and eukaryotes (Hsa RNase H2) depicts a conserved catalytic core, termed RNase H fold, consisting of five-stranded β sheet with three antiparallel and two parallel strands (5α123, ↑↑↓↑) surrounded by α-helices (Figure 4B and 4C). When Tko RNase HII was superimposed on the structure of Hsa RNase HII using the positions of 140 pairs of C-α atoms, the resulting root mean square deviation (RMSD) was 1.278 Å (Figure 4B). In this model, the secondary elements superimpose very well. The active site geometry and the conserved tyrosine residue (Y210 in H. sapiens and Y170 in T. kodakarensis) strikingly overlap. This suggests that the cleavage mechanism is conserved in eukaryotes and Archaea. When Hsa RNase HII was superimposed to the structure of the Tma RNase HII substrate complex, using the positions of 128 pairs of C-α atoms, the resulting root mean square deviation (RMSD) was 2.414 Å (Figure 4C). In such a superimposition, the RNase H fold overlaps quite well and the active-site residues adopt a similar geometry as also observed with the archaeal orthologue, suggesting that a two-metal ion catalytic mechanism operates in RNases HII/2. However, the spatial clustering of residues surrounding the conserved tyrosine (Y163 in T. maritima) is clearly different between the bacterial and eukaryotic/archaeal RNases HII/2. Indeed, a bulky secondary structural element, consisting of a short one-turn helix (α7 in T. maritima at the top of the secondary structure alignment; Figure 4A and α7 in magenta; Figure 4C), locates in close proximity of the active site in the bacterial structure. This region, in the archaeal/eukaryotic structures, corresponds to a linear straight segment in which an aspartic residue points toward the cleaved DNA_1RNA_DNA strand (Figure 4C). No counterpart acidic residue is found in the bacterial structure. Therefore, these subtle structural differences may affect the spatial positioning of essential amino acids for cleavage.

Discussion

A frequent phenomenon, having profound, detrimental effects on genome stability of both prokaryotes and eukaryotes, is the failure to remove rNMPs from DNA (8). In Bacteria, including Archaea, as well as in mammalians and in yeast cells, the main processing mechanism responsible for repairing these lesions in DNA is the ribonuclease excision repair (RER) pathway, which involves the RNase H type 2 enzyme (15, 18, 24, 31, 32). Similarly to deoxyribonucleotides, rNMPs are susceptible to modifications and oxidative insults (33, 34). Moreover, a significant generation of abasic site formation has been demonstrated upon RNA oxidation and alkylation (35). Therefore, among the many rNMPs that are introduced in the nuclear genome per cell cycle (36), a significant amount of incorporated rNMPs could be oxidized or become abasic sites, thus affecting genome stability. In a recent work, we found that eukaryotic RNases H2 derived from human, mouse and budding yeast were unable to process rAP or oxidized rNMP embedded in DNA, which, on the contrary, were readily leaved by the APE1 protein of the base excision repair (BER) pathway (20). Here, we evaluated whether rAP or oxidized rNMPs embedded in DNA could be processed by the prokaryotic orthologous of RNase H type 2 proteins. We demonstrated that while bacterial RNase HII from E. coli was proficient in processing both oxidized rNMP and rAP embedded in DNA, in a Mg2+-dependent manner, the archaeal orthologue from P. abyssi was unable to incise at either of these sites (Figure 5). Interestingly, Cilli et al., (9) showed that the commercial prokaryotic RNase HII has the ability to remove a single rNMP when paired to an oxidized base or to incise an oxidized rNMP in a DNA duplex. Moreover, Sassa et al., (37) showed that the commercially available prokaryotic E. coli RNase HII preserves the ability to remove an...
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oxidized rNMP in a DNA duplex. In line with these studies, our present findings confirm that commercially available E. coli RNase HII retains the ability to process both rAP and oxidized rNMPs embedded in DNA, while eukaryotic RNase H2 is completely inactive on these substrates (20). Our data suggest that the ability to process abasic residues or r8oxoG embedded in DNA might have been lost during evolution. We also noticed that the ability of E. coli RNase HII to process the rNMPs embedded in DNA does not absolutely depend on base pairing, as demonstrated by the fact that the rG is processed significantly more efficiently when it is paired with dA than with dC. Interestingly, the human RNase H2 protein retains some preference for cleavage of rG:dA versus rG:dc (20), although not as prominent as observed for E. coli RNase HII (Figure 2A and 2B). Archaeal RNase HII from P. abyssi has undetectable ability to cleave rG opposite to dA, while it cleaves the canonical rG:dc pair (Figure 3B and 3C). It is possible that the rG:dc pair causes a local helix distortion, which may facilitate protein recognition of the lesion in the bacterial and marginally in the eukaryotic form of RNase H type 2. It will be interesting to investigate whether the archaenal mismatch repair system can recognize and process mispaired rNMPs in DNA as the E. coli and S. cerevisiae MMR factors do (14). Our findings clearly reveal the inability of archaenal RNase HII to incise at oxidized rNMPs or at abasic residues and are similar to results obtained using eukaryotic RNases H2 from budding yeast, mouse and human (20), but contrary to those obtained using E. coli RNase HII. The differences between bacterial RNase HII and archaenal/eukaryotic RNases HII/2 in their capacity to cleave rAP and oxidized rNMP embedded in DNA might be linked to different positioning of residues surrounding the active site. In our study, archaenal/eukaryotic RNases HII/2 were able to bind both oligonucleotides containing embedded abasic and oxidized residues in dsDNA like the bacterial counterpart (Figure 3F). Despite the overall structural conservation between archaenal, bacterial and eukaryotic RNases HII/2, we demonstrate that specific ribonucleotide enzyme catalysis has not been similarly preserved among Bacteria, Archaea and Eukarya. Our results are in agreement with previous studies, showing differential substrate preferences between bacterial and archaenal/eukaryotic RNases HII/2 (28, 29). Although the precise evolutionary relationship between eukaryotes and Archaea continues to be a subject of debate (38), these findings clearly highlight a common evolutionary scenario between Archaea and eukaryotes in keeping inactive RNases H/2 on modified rNMPs embedded in DNA. Inability to process modified rNMPs by the RER mechanism might have led to alternative removal pathways. In eukaryotes, embedded abasic residues and oxidized rNMPs in dsDNA can be processed by APE1 (20). Moreover, damage tolerance pathways involving translesion DNA polymerases can be employed to prevent the stalling of DNA replication at difficult lesions in eukaryotic cells (37). It is commonly accepted that genetic traits are gained by duplication, which permits separate evolution into two functions, as a consequence of a selective pressure in favor of a new trait which gives an adaptive advantage to the organism and/or because of the lack of pressure to retain that trait. In Archaea, the observed absence of ability of RNase HII to cleave rAP and oxidized rNMP sites might be explained on the basis of adaptive molecular mechanisms to survive at extremely high temperatures. Moreover, we cannot exclude, at present, that an interacting partner might render P. abyssi RNase HII or similarly eukaryotic RNase H2 active, or, on the other hand, another protein is involved in this process. In light of our previous work (20), showing that APE1 is, in eukaryotic cells, able to actively cleave rAP- and, to a lesser extent, r8oxoG- containing substrates, some important questions arise, including whether the APE1 pathway could be less error prone than the RER pathway and what could be the biological advantage.

More investigations are now required to elucidate these issues in both archaenal and mammalian cells to further support the hypothesis that the mechanisms observed could be correlated with evolutionary processes.

Experimental procedures
Double-stranded synthetic oligonucleotides

All unmodified and modified double-stranded oligonucleotides (ODNs) sequences are described in Table 1. The rNMP-containing 40-mer, ss_rG_40 and ss_rF_40, were purchased from Dharmacon (Colorado, USA) and annealed with complementary sequence, ss_comp_40, purchased from Invitrogen (New York, USA). The oligos ss_rG_40 and ss_rF_40 were 5' end-labeled with [γ-32P] ATP (PerkinElmer, Massachusetts, USA) by T4 polynucleotide kinase (PNK) (New England BioLabs, Massachusetts, USA) in a reaction mixture containing 10 μM ATP using 10X PNK buffer (New England BioLabs, Massachusetts, USA). This labeling reaction was carried on at 37 °C for 1 hour, followed by
inactivation at 65 °C for 20 minutes. The reactions were purified by using Illustra MicroSpin G-25 column (GE Healthcare, Buckinghamshire, UK). ss_dG, ss_rG, ss_d8oxoG ODNs, labelled with IRDye700 fluorophore at 5’ end, and their complementary strand, ss_comp, were synthesized from Metabion International AG (Steinkirchen, Germany). In parallel, ss_df probe was synthesized from IDT Technologies (Coralville, USA) and labelled with IRDye800 fluorophore at 5’ end. Finally, 1’ rOH ODN, called ss_roH, was synthesized from Dharmacon (Colorado, USA) and labelled with Cy5 fluorophore at 5’ end (Table 1). All ODNs were purified by Reverse Phase – High Performance Liquid Chromatography (RP-HPLC). Finally, synthesis of the oligonucleotide containing an internal ribose 8-oxo-guanosine (r8oxoG) and an IRDye700 fluorophore at 5’ end was in-house carried out as already described (20, 39). For performing the annealing reaction, all ssODNs were re-suspended in RNase- and DNase-free water at 100 µM. Then, 100 pmol of each probe was annealed with 150 pmol of its complementary DNA strand (as indicated in Table 1) in 10 mM TrisHCl pH 7.4 and 10 mM MgCl₂, heated at 95°C and cooling down over night in the dark.

Expression and quantification of recombinant proteins

Recombinant *E. coli* RNase HII was purchased from New England BioLabs®. Recombinant *Pyrococcus abyssi* (Pab) RNase HII was produced as described in Le Laz *et al.* (22). Different amounts of *Pab* RNase HII were loaded onto a 10 % (w/vol) sodium dodecyl sulphate-polyacrylamide (SDS-PAGE) electrophoresis gel, which was subsequently stained using Coomassie Brilliant Blue stain (ThermoFisher, Waltham, MA, USA) and visualized with an Odyssey CLx Infrared Imaging system (LI-COR GmbH, Germany). The signal of each band was quantified and normalized with a BSA (bovine serum albumin) standardization curve using Image Studio software (LI-COR GmbH, Germany) (Figure 3A). Recombinant human RNase H2 was produced as described in Chon *et al.* (24, 40) and tested for its activity and purity in our previous publication (20).

RNase HII activity assays

All ³²P labeled substrates (10 nM) were treated with 1 U of *E. coli* RNase HII enzyme (New England BioLabs®, Massachusetts, USA) for 1 hour at 37 °C in 10 X Thermopol® buffer (New England BioLabs, Massachusetts, USA). This was followed by stopping the reaction by adding 2 X denaturing polyacrylamide gel electrophoresis (PAGE) gel buffer (0.01 % bromophenol blue, 95 % formamide and 20 mM EDTA pH 8.0) and heating to 95 °C for 5 minutes. After dilutions, the products of this reaction were analyzed by 15 % (wt/vol) polyacrylamide, 8 M urea gel electrophoresis (urea-PAGE). 20-100 Oligonucleotide Length Standard (Integrated Device Technology, Iowa USA) was used as a ladder. After electrophoresis, gels were exposed to phosphor screen overnight. Images were taken with Typhoon Trio+ (Colorado, USA) and obtained with ImageQuant (GE Healthcare). Band intensities were quantified by Multi Gauge V3.0 (Fujifilm). Endoribonuclease activity of *E. coli* RNase HII on 25-mer dsODNs was investigated by reacting 1 U (0.1 ng/µl) of the protein with 250 fmol of DNA or RNA/DNA oligonucleotides (25 nM) in the commercial 10 X ThermoPol® Reaction Buffer (New England BioLabs® Inc, Milan, Italy) or in the equivalent home-made buffer containing 10 mM KCl, 20 mM TrisHCl pH 8.8, 10 mM (NH₄)₂SO₄, 2 mM MgSO₄, 0.1 % Triton X-100, in a final volume of 10 µl for the indicated times at 37 °C. Endoribonuclease activity of *P. abyssi* RNase HII on 25-mer dsODNs was tested by reacting 200 fmol (20 nM) of the protein with 250 fmol of DNA or RNA/DNA oligonucleotides (25 nM) in a buffer containing 50 mM TrisHCl pH 8.0 and 5 mM MgCl₂ in a final volume of 10 µl for the indicated times at the optimal temperature of 60 °C. At the end of all reactions, samples were blocked with a stop solution, containing 99.5 % vol/vol Formamide (SIGMA-ALDRICH, Milan, Italy) and 10 X Orange Loading Dye (LI-COR Biosciences, Milan, Italy) and heated at 95°C for 5 minutes. Then, all samples were loaded onto a 7 M denaturing 20 % polyacrylamide gel and were visualized with an Odyssey CLx Infrared Imaging system (LI-COR GmbH, Germany). The signals of the non-incised substrate (S) and the incision product (P) bands were quantified using Image Studio software (LI-COR GmbH, Germany).

Cell culture, genomic RNA extraction and Ribonuclease activity assay

HeLa cells (from ATCC®) were cultured in DMEM (EuroClone, Milan, Italy), supplemented with 10 % (vol/vol) fetal bovine serum (EuroClone, Milan, Italy), 100 U/ml penicillin, 100 mg/ml streptomycin sulphate and 2 mM l-glutamine (EuroClone, Milan, Italy) at 37 °C and 5 % CO₂. Total RNA from HeLa cells was extracted with the SV Total RNAisolation System kit (Promega,
Madison, WI). RNase A enzymatic assay was performed by incubating 400 ng of total RNA with 0.1 ng of Ribonuclease A (RNase A) from bovine pancreas (SIGMA-ALDRICH, Milan, Italy). Prokaryotic RNase HII enzymatic assay was performed incubating 400 ng of total RNA with increasing amounts of *E. coli* RNase HII. Reactions were carried on 1 hour at 37 °C in a final volume of 10 µl. Upon adding 2 X RNA Loading Dye (ThermoFisher, Waltham, MA, USA), samples were heated at 65 °C for 5 minutes and then, moved on ice. Samples were run in a 1 % (w/vol) agarose gel, prepared by thawing 1 g of RNase-free agarose powder (SIGMA-ALDRICH, Milan, Italy) in 85 ml of MOPS 1 X (20 mM MOPS, 10 mM Sodium acetate, 1 mM EDTA) and 15 ml of Formaldehyde solution (SIGMA-ALDRICH, Milan, Italy). The run was carried on in Buffer MOPS 1 X, at 3-4 V/cm. Gel were visualized with Gel Doc 2000 (Biorad, Milan, Italy).

**Crosslinking analyses**

20 pmol of human RNase H2 (2 µM) was co-incubated with 250 fmol of the probe (25 nM) for 30 minutes at 37 °C in a buffer containing 10 mM TrisHCl pH 7.4, 25 mM KCl, 1 mM MgCl₂ and 10 mM EDTA in a final volume of 10 µl. Similarly, 20 pmol of *P. abyssi* RNase HII (2 µM) was co-incubated with 250 fmol of the probe (25 nM) for 30 minutes at 60 °C in a buffer containing 50 mM TrisHCl pH 8.0, 5 mM MgCl₂ and 10 mM EDTA in a final volume of 10 µl. After binding reaction, samples were exposed to 0.2 J/cm² UV rays and then stopped in Laemmli 4X, heated at 95°C for 5 minutes and loaded onto an 8 % (w/vol) SDS PAGE gel. After the run, gel was visualized with an Odyssey CLx Infrared Imaging system (LI-COR GmbH, Germany).

**Statistical analysis**

Statistical analyses were performed by using the Student’s t-test in GraphPad Prism software. When P<0.05, data were considered as statistically significant.

**Acknowledgments**

The authors thank the Tell and Storici laboratories for constructive feedbacks during the development of this work.

**Conflict of interest**

The other authors declare that they have no conflicts of interest with the contents of this article.

**Author contributions**

G.T. designed and conceived the study and supervised the experiments with contribution from F. S., G. H. and R.C.; M.C.M. performed most of the experiments, analyzed the data and critically contributed to the interpretation of the results; G.H. performed sequence alignments and structural modeling; S. B., K.D.K. and G.N. contributed to experiments using *E. coli* RNase HII; G.T., M.C.M., F.S., and G.H. mainly wrote the manuscript. All authors critically read and approved the final version of the manuscript.
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Footnotes

This work was supported by grants from Associazione Italiana per la Ricerca sul Cancro (AIRC) [grant number IG19862] to G. Tell, by the National Institutes of Environmental Health Sciences (NIEHS) of the NIH [grant number 1R01ES026243-01] and the Howard Hughes Medical Institute Faculty Scholar [grant number 55108574] to F. Storici.

The abbreviations used are: rAP, ribose monophosphate abasic (both apurinic or apyrimidinic) site; r8oxoG, oxidized ribonucleoside monophosphate; RER, ribonucleotide excision repair; RNase HII/2, type 2 ribonuclease H; ODNs, oligonucleotides.
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| ss_rG_40  | dAdGdTdTdTdGdAdTdTdTdTdTdAdNdGdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdTdT
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Figure Legends

Figure 1. RNase HII from E. coli cleaves rAP embedded in dsDNA
(A) Scheme of the ss and ds 40-mer substrates used to test the activity of E. coli RNase HII including a riboabasic site mimicked by a tetrafururan residue indicated with rF and an unmodified rGMP (rG) embedded in DNA. DNA strands are depicted in blue, whereas rNMPs in red. The 5’ radiolabels, indicated by 32P, at the 5’ is in grey. (B) Representative denaturing polyacrylamide gel of cleavage result obtained on all substrates without (lanes 1-6) and with (lanes 7-12) E. coli RNase HII protein. First left lane, indicated as “M”, corresponds to ssDNA ladder. On the left side of the image molecular weights are indicated with an arrow to the corresponding bands. Lanes 1, 7 and 4, 10 are ss substrates containing rG and rF, respectively; whereas lanes 2, 8 and 5, 11 are ss substrates containing rG and rF, respectively, which is cooled slowly at room temperature to observe any self-annealing; lanes 3, 9 are ds substrates containing rG. Lanes 6, 12 are ds substrates containing rF. The cleavage percentages of reactions are displayed below the image.

Figure 2. RNase HII from E. coli exclusively cleaves rAP and oxidized rNMP embedded in DNA
(A) Representative denaturing polyacrylamide gel of incision by E. coli RNase HII on different 25-mer dsODNs. Endoribonuclease activity of E. coli RNase HII protein was investigated in vitro at different time points using the commercial ThermoPol® Buffer as specified in Materials and Methods section. ds_dG:dC and ds_d8oxoG:dC were used as negative control ODNs, whereas ds_rG:dC was used as positive control. Moreover, rG and r8oxoG containing ODNs were tested in two different pairing conditions: paired with dC or with dA. “S” denotes substrate position and “P” denotes product position. Time points, expressed in minutes, are shown on the top of the figure. Alongside each panel, a schematic representation of each substrate is reported. dsODNs are symbolized as blue lines standing for 25-mer in which the 13th base on the 5’ strand is a deoxy- or ribo-modified site paired with a base (dC or da) on the complementary strand (3’→ 5’). If the modified site is a deoxyribonucleotide (d), it was colored in blue; whereas if this is an rNMP (r), it is colored in red. Lastly, a fluorophore IRDye700, IRDye800 or Cy5 dye labels the top 5’ end of each dsODN. (B) Graph shows the time course kinetics of E. coli RNase HII incision on each substrate. The activity is reported as percentage of substrate converted to product at the indicated time. Data are expressed as mean ± SD of three independent replicates. p value of < 0.001 is marked as two asterisks (***). (C) Representative RNase-free agarose gel, in which genomic RNA samples extracted from HeLa cells were run after incubation with both ribonucleases, RNase A and E. coli RNase HII, as explained in Materials and Methods. On top of the image, the absence or presence of the ribonuclease enzymes is indicated as “-” or “+”, respectively. Specifically, the two different amounts of E. coli RNase HII were indicated on top of the figure, expressed in units (U). 28S and 18S rRNAs are pointed at the left of each corresponding band. RNA degradation upon RNase A treatment is indicated by an arrow on the right side of the figure. (D) Representative denaturing polyacrylamide gel shows the activities of RNase HII (1 U) and RNase A (0.1 ng) on ds_rOH:dC, ds_rG:dC, ds_dF:dC and ds_r8oxoG:dC oligonucleotides after 1-hour incubation at 37°C. At the left of the panel, “S” and “P” indicate the substrate and the product, respectively. (E) Representative denaturing polyacrylamide gel of time course kinetics cleavage by a fixed amount of E. coli RNase HII on ds_rG:dC and ds_r8oxoG:dC oligonucleotides under different time points, expressed in minutes and indicated on the top of the figure. The reaction was performed in the home-made ThermoPol® Buffer as explained in Materials and Methods section. The MgSO4 (2 mM) effect was investigated removing its presence from the buffer. Moreover, in the samples in which the MgSO4 was removed, EDTA (5 mM) was added. “S” and “P” correspond to substrate and product, respectively. (F) Relative graph illustrating the time-course kinetics activity. Data are expressed as mean ± SD of three independent replicates.

Figure 3. RNase HII from P. abyssi does not cleave rAP and oxidized rNMP embedded in DNA
(A) Different volumes (1 and 2 µl) of the recombinant RNase HII protein from P. abyssi were quantified as described in Materials and Methods. Both bands corresponding to different amounts of the protein (apparent Mw: 27’600 Da) were quantified and normalized on a standardization curve (250-500-750 ng) of Bovine Serum Albumin (BSA) protein. The molecular weight (Mw) expressed in kilo Daltons (kDa) is shown on the left side of the image. (B) Representative denaturing polyacrylamide gel of time course kinetics cleavage by a fixed amount of Pab RNase HII protein on each substrate. Different time points, expressed in minutes, are indicated on the top of the figure. ds_rG:dC oligonucleotide was used as positive control. “S” and “P” correspond to substrate and product, respectively. (C) Relative graph illustrating the time-course kinetics
activity of the protein on different ODNs. Data are expressed as mean ± SD of three independent replicas. p value of < 0.001 is marked as two asterisks (**). (D) Representative denaturing polyacrylamide gel of temperature-response cleavage by Pab RNase HII on each ODN. The reaction was performed for 15 minutes at different temperatures indicated on the top of the figure and expressed in “°C”. “S” and “P” correspond to substrate and product, respectively. (E) Relative graph illustrating the Pab RNase HII cleavage activity upon different temperatures on all the used dsODNs. Data are expressed as mean ± SD of three independent replicas. p value of < 0.001 is marked as two asterisks (**). (F) RNases HII/2 from human and Pab recognize oxidized rNMP and rAP embedded in DNA, though being unable to process them. After the incubation between dsODNs and human or Pab type 2 RNase H proteins, binding complexes were cross-linked upon UV exposure as explained in Materials and Methods section, and run on a denaturing 8 % (w/vol) SDS–PAGE gel. Bands correspond to the complex DNA-protein, migrating in a different way following the molecular weight (Mw) of both proteins and dsODNs. Mw, expressed in kilo Daltons (kDa), is shown on the right side of the image.

Figure 4. Type 2 RNases H from Archaea and eukaryotes display the highest structural similarities. (A) Structure-base sequence alignment of Thermotoga maritima (Tma), Thermus thermophilus (Tth), Escherichia coli (Eco), Saccharomyces cerevisiae (Sce), Homo sapiens (Hsa), Mus Musculus (Mmu), Archaeoglobus fulgidus (Afu), Pyrococcus abyssi (Pab) and Thermococcus kodakarensis (Tko). Sequence alignment is based on the three-dimensional structure of RNase HII_Tma (corresponding secondary structure elements are shown at the top) (PDB: 3O3F) and RNase HII_Tko (corresponding secondary structure elements are shown at the bottom) (PDB: 1IO2). Conserved active site residues are marked with black dots. Strictly conserved residues are highlighted in red. Structure-base sequence alignment was generated using ESPript 3. (B) Superposition of structures of RNase HII/2 from T. kodakarensis (PDB: 1IO2) in yellow and H. sapiens (PDB: 3PUF) in green on the C-α atoms from the central β-sheet of the RNase H fold. Active site residues in red and the conserved tyrosine residue are shown as sticks. The two conserved α-helices (α1 and α5 secondary elements from RNase HII_Tko depicted at the bottom of the alignment) in T. kodakarensis and H. sapiens but absent in T. maritima are encircled. (C) Superposition of structures of RNase HII/2 from T. maritima (PDB: 3O3F) in blue and H. sapiens (PDB: 3PUF) in green on the C-α atoms from the central β-sheet of the RNase H fold. Active site residues in red and the conserved tyrosine residue are shown as sticks. ds_DNA_1RNA_DNA is shown as orange lines excepted for the 1RNA which is shown as black sticks. Magnesium ions are shown as pale pink spheres. The unique magenta α-helice (α7 secondary elements from RNase HII_Tma depicted at the top of the alignment) is encircled. The arrow indicates the missing aspartic residue in T. maritima. Superpositions and structural figures were prepared in PyMol (Schrödinger).

Figure 5. Difference in cleavage activity of modified ribonucleotides embedded in DNA among RNase HII/2 from the indicated species. The endoribonuclease activities of the indicated enzymes, expressed as percentage of cleavage, on each of the substrates used in our study, are represented as a heat map, in which grey color represents lack of any enzymatic activity while the different colours from green to red, represent increasing processing activity. Specifically, in the case of E. coli activity, the percentage of cleavage obtained from data shown in Figure 2 was considered; in the case of P. abyssi activity, the percentage of cleavage obtained from data shown in Figure 3 was considered; in the case of human RNase H2 activity, the percentage of cleavage obtained from data published in Malfatti et al., 2017 was considered. Experimental conditions for each enzyme were the same when testing the different substrates.
Figure 1. RNase HII from *E. coli* cleaves rAP embedded in DNA.
Figure 2. RNase HII from *E. coli* exclusively cleaves rAP and oxidized rNMP embedded in DNA.
Figure 3. RNase HII from *P. abyssi* does not cleave rAP and oxidized rNMP embedded in DNA
Figure 4. Type 2 RNases H from Archaea and eukaryotes display the highest structural similarities
Figure 5. Difference in cleavage activity of modified ribonucleotides embedded in DNA among RNase HII/2 from the indicated species.
Unlike the E. coli counterpart, archaeal RNase HII cannot process ribose monophosphate abasic sites and oxidized ribonucleotides embedded in DNA.

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*J. Biol. Chem.* published online July 12, 2019

Access the most updated version of this article at doi: 10.1074/jbc.RA119.009493

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