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Biochemical and Serological Evidence for an RNase E-Like Activity in Halophilic *Archaea*

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Endoribonuclease RNase E appears to control the rate-limiting step that mediates the degradation of many mRNA species in bacteria. In this work, an RNase E-like activity in *Archaea* is described. An endoribonucleolytic activity from the extreme halophile *Haloarcula marismortui* showed the same RNA substrate specificity as the *Escherichia coli* RNase E and cross-reacted with a monoclonal antibody raised against *E. coli* RNase E. The archaeal RNase E activity was partially purified from the extreme halophilic cells and shown, contrary to the *E. coli* enzyme, to require a high salt concentration for cleavage specificity and stability. These data indicate that a halophilic RNA processing enzyme can specifically recognize and cleave mRNA from *E. coli* in an extremely salty environment (3 M KCl). Having recently been shown in mammalian cells (A. Wennborg, B. Sohlberg, D. Angerer, G. Klein, and A. von Gabain, Proc. Natl. Acad. Sci. USA 92:7322–7326, 1995), RNase E-like activity has now been identified in all three evolutionary domains: *Archaea*, *Bacteria*, and *Eukarya*. This strongly suggests that mRNA decay mechanisms are highly conserved despite quite different environmental conditions.

Biochemical studies have led to the division of living organisms into three domains: *Eukarya*, *Bacteria*, and *Archaea* (11, 41). Members of the domain *Archaea* are notable for the extreme ecological niches which they occupy (hyperthermal, hypersaline, anaerobic, hyperbaric, and extreme pH) and for their particular characteristics. As more and more molecular mechanisms are studied in members of the domain *Archaea*, it is becoming apparent that they share deep similarities with eukaryotes. The most striking one is the similarity in transcription machinery between eukaryotes and archaebacteria (for a review, see reference 18). In eubacteria and eukaryotes, the control of mRNA stability represents an important contribution to gene expression (17, 28, 35). The regulation of mRNA half-life in eubacteria is critical for rapid response to changes in the environment (30, 33). However, RNA decay mechanisms have been well studied only in *Escherichia coli*, where the degradation of mRNA has been shown to be mediated by a combination of specific endonucleolytic cleavages followed by exonucleolytic degradation (1). RNase E has been proposed as the indispensable endoribonuclease that initiates most mRNA decay processes. Genetic studies suggest that a general alteration of mRNA decay is associated with defective RNase E. Furthermore, in vivo and in vitro studies have shown that RNase E is necessary for the specific cleavage of many mRNA molecules (for a review, see reference 25). Interestingly, for some of these specific processing reactions, RNase E cleavage activity has been shown to be regulated by environmental changes (15, 19) and it is believed that the action and specificity of RNase E may be modulated by binding to a number of proteins, such as exonucleases (6), helicases (32), and chaperonins (GroEL, DnaK) (27). RNase E has been found in bacteria other than *E. coli* (14, 38). An RNase E-like activity was recently found in human cells, and a gene product of human origin has been shown to complement RNase E deficiencies in *E. coli* (39, 40). This endoribonucleolytic activity processes *E. coli ompA* mRNA and 9S RNA with the same specificity as RNase E from *E. coli*. These data led to the idea that RNase E might be a key enzyme for control of gene expression, which has been highly conserved in evolution. Along with RNase E, RNases P and III have been identified in domains other than *Bacteria*: RNase P in both *Eukarya* and *Archaea* and RNase III in *Eukarya* (20, 22, 29). Except for a few studies, little is known about RNA processing and mRNA decay in *Archaea* (22). The organization of ribosomal genes has been examined in several *Archaea* species and, in most cases, appears to be similar to that in *Eubacteria* in that the genes are linked in the order 16S-23S-5S and transcribed from a common promoter. A recent study of a hyperthermophile has shown the involvement of an endoribonuclease involved in rRNA processing (16). To investigate the possible evolutionary conservation of mRNA processing and decay mechanisms in all living cells, we have tested for the existence of RNase E in the *Archaea* domain by using the halophilic archaeon *Haloarcula marismortui*. Such organisms balance an extreme external salt concentration (4 M NaCl) by accumulating multimolar KCl in their cytoplasm (12). Thus, proteins from halophilic organisms are adapted to be stable and soluble and to function at an extremely high salt concentration. By using an in vitro processing system, we found that different RNA species with well-characterized cleavage sites for *E. coli* RNase E were cleaved in the same manner by archaeal extracts or by bacterial RNase E. This endoribonuclease activity was partially purified and shown to contain polypeptides that are serologically related to *E. coli* RNase E. The stability and specificity of the enzyme at different salt concentrations were also studied.

MATERIALS AND METHODS

***Archaea* strains and plasmids.** *Haloferax volcanii* WFD 11 was obtained from W. F. Doolittle (University of Halifax). *Halobacterium halobium* S9 was provided by D. Oesterhelt (Max-Planck-Institut, Martinsried, Germany). *H. marismortui* ATCC 43049 was a gift from K. Nierhaus (Max-Planck-Institut, Berlin, Germa-

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ny). *E. coli* CAN 20-12E/18-11 (38) and the human B-cell line BJAB were used for the preparation of P-100 extracts. *E. coli* BL21 (DE3) transformed with the pRE296 plasmid was kindly provided by A. Miczak (27). This strain overexpresses RNase E after isopropyl-β-D-thiogalactopyranoside (IPTG) induction.

Plasmid pT7ompA+5* contains the 5′ untranslated region (UTR) from *ompA* (8), and plasmid pTH90 harbors the sequence for 9S RNA (37). A T4 gene 32 DNA fragment with a T7 promoter was obtained by PCR amplification of pTAK65 (5) with the primers PR1 (5′-GTG GTA AGC TTC ATT TTT ATT TCC TTT TTA ATT TAA TTT AA-3′) and PR2 (5′-GTG GAA TTC CTA ATA CGA CTC ACT ATA GGC CTC TCG AAG ACC CAG AGT ATT GC-3′).

Preparation of cell extracts and protein purification. Human and *E. coli* P-100 extracts were prepared as described previously (40). For primer extension experiments, a protein extract enriched in RNase E activity was prepared from the RNase E-overexpressing strain. The clarified cell lysate was prepared as described by Miczak et al. (27) and precipitated with ammonium sulfate (40% saturation). The pellet was resuspended in TBS buffer (50 mM Tris-HCl, 150 mM NaCl, pH 7.4) containing 10% glycerol and stored at −20°C until use.

For preparation of archaeal crude cell extracts, cells were cultivated aerobically in an Erlenmeyer flask with gentle agitation in halophilic media containing 4 M NaCl. *H. halobium* was grown at 39°C as previously described (31). *H. marismortui* and *H. volcanii* were grown at 37°C as previously described (26). A 50-ml volume of a cell suspension (A_{280}, 1) were spun at 4,000 × *g*. The cell pellet was resuspended in 1 ml of ice-cold lysis buffer containing 50 mM Tris-HCl (pH 8), 2 M KCl, 50 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol (DTT), 0.1 mM $ZnCl_2$, 10% glycerol, and 1 mM phenylmethylsulfonyl fluoride. The cells were disrupted on ice by sonication (six times for 10 s each time), and the cell debris were separated by centrifugation at 12,000 × *g* and 4°C for 5 min. The fresh supernatant was used for processing assays. To obtain *H. marismortui* total, polysomal, and nonpolysomal fractions, 3 g of cell paste was resuspended in 5 ml of lysis buffer containing 3 M KCl, 0.1 M NH_4Cl, 20 mM magnesium acetate [$Mg(OAc)_2$], 40 mM Tris-HCl (pH 7.6), 1 mM DTT, and 1 mM phenylmethylsulfonyl fluoride. After sonication, the lysate was clarified by centrifugation. Polysomes were pelleted at 100,000 × *g* for 1 h in a Beckman TL-100 ultracentrifuge and resuspended in a volume of lysis buffer equivalent to that of the supernatant. For partial purification of the RNase E activity from *H. marismortui*, cells were grown at 40°C in a 20-liter fermentor (Infors) with oxygenation at 4 liters/min and agitation at 300 rpm. The pink-red cells were harvested by centrifugation at 5,000 × *g* for 15 min and stored at −80°C until used. Sixty-five grams of cells was thawed in 2 volumes of buffer A [2.2 M $(NH_4)2SO_4$, 50 mM Tris-HCl (pH 7.6), 40 mM $Mg(OAc)_2$, 1 mM DTT) with one protease inhibitor cocktail tablet from Boehringer. After disruption by sonication (10 times for 30 s each time at maximum strength), the cell debris fraction was separated by centrifugation at 37,000 × *g* for 45 min at 4°C. Macromolecular components were removed by centrifugation at 160,000 × *g* for 4 h in a Beckman Ti 70 rotor. The S-160 clear supernatant was dialyzed against buffer A overnight at 4°C and then applied to a 40-ml DE-52 ion-exchange column (Whatman) preequilibrated in buffer A at room temperature. The column was washed with 5 column volumes of buffer A. Bound proteins were fractionated in a 400-ml gradient from buffer A to buffer B [0.5 M $(NH_4)2SO_4$, 2 M NaCl, 50 mM Tris-HCl (pH 7.6), 40 mM $Mg(OAc)_2$]. Protein concentration was determined as described by Bradford (3), with the dye reagent from Bio-Rad. For Western blot analysis, proteins from the different extracts were precipitated by 20% trichloroacetic acid. The protein pellets were washed with cold acetone, resuspended in a 20 mM Tris-base solution, and boiled for 5 min in a Laemmli sample cocktail (31) containing 6 M urea. Equal amounts of protein from each of the DE-52 column fractions were loaded on a sodium dodecyl sulfate–12% polyacrylamide gel (31). After electrophoresis, the gels were blotted onto Hybond polyvinylidene difluoride (Amersham). The membranes were probed with a monoclonal antibody which is reactive against *E. coli* RNase E. The antibody, G144-25.2, was generated in baculovirus against the Rne segment encoded by the *Afl*III fragment (7, 23a). Detection was performed with the enhanced-chemiluminescence detection system from Amersham.

In vitro transcription, RNase E activity assay, and primer extension analysis. The RNA substrates were transcribed from linearized plasmids or from PCR products with T7 RNA polymerase in the presence or absence of [α-^{32}P]UTP and purified on a denaturing polyacrylamide gel. RNase E processing was assayed essentially as previously described by using 20 μg of yeast tRNA as a nonspecific competitor (40). For halophilic activity assays, increasing amounts of KCl (up to 3 M) were added to the standard reaction. A 50-μg sample of archaeal crude extract or 10 μg of an ion-exchange column fraction was used per assay. After incubation for 1 h at 37°C, the reaction was terminated by phenol extraction, followed by ethanol precipitation. The RNA pellets were extensively washed with 70% ethanol to remove salts. The radioactive RNA cleavage products were resolved on a 6% denaturing polyacrylamide gel.

The RNase E-specific cleavages in the *ompA* RNA were mapped by primer extension with the *nru*I-1 primer (5′-CGATAGCTGTGTTTTTGATTTTTTGC GCC-3′) (23). Unlabeled substrate (0.2 pmol) was incubated with protein extract from the *E. coli* RNase E-overexpressing strain (0.5 μg) or *H. marismortui* total protein extracts (50 μg) and purified as described above. The digested RNA was analyzed by primer extension as previously described (24), except that actinomycin D (50 μg/ml) and 10 U of RNase inhibitor (Boehringer) were added to the elongation mixture. Plasmid pT7ompA+5* was primed with the *nru*I-1 primer and sequenced by the dideoxynucleotide chain termination method (36). Primer extension and sequencing products were run in parallel on an 8% polyacrylamide gel containing 7 M urea. Amersham Hyperfilm MP was used for all autoradiography.

RESULTS AND DISCUSSION

Processing of RNase E substrates in the same manner in archaeal eubacterial, and eukaryotic extracts. The 5′ UTR of *ompA* mRNA from *E. coli* was used to assay for RNase E-like activity in cellular extracts. When the in vitro-transcribed, ^{32}P-labeled 5′ UTR of *ompA* mRNA was incubated with extracts from three different halophilic archaea (*H. marismortui*, *H. volcanii*, and *H. halobium*) or with extracts from *E. coli* or the human cell line BJAB, similar processing patterns were obtained (Fig. 1A). This endoribonuclease activity was recovered when proteins of an S-160 supernatant from *H. marismortui* were fractionated by ion-exchange chromatography. The two major processing products correspond in size to the two expected upstream cleavage products, C and D, generated by RNase E (23). In assays with extracts from *E. coli* or BJAB, the additional bands are due to partial digestion products and differences in exonucleolytic activities. These complex processing patterns have also been reported with highly purified RNase E from *E. coli* (6). The fact that we detected only the upstream cleavage products is most likely due to contaminating exoribonucleases that attack the *ompA* downstream cleavage products, which are less protected by secondary structures (34). To show that the *ompA* processing products are indeed due to an RNase E-like endoribonuclease activity, we mapped cleavage sites by primer extension. Figure 1B shows that a cleavage site that corresponds to the RNase E-specific D cleavage (GU/AUUUU) was detected. Thus, cleavage product D is a true result of endoribonuclease cleavage. The C cleavage signal was also detectable, although it was weak.

To investigate the ability of the archaeal endoribonuclease activity to cleave other specific RNase E targets, we tested 9S RNA from *E. coli* containing a high degree of secondary structures. RNase E was originally defined as the endoribonuclease that cleaved the rRNA processing intermediate 9S into p5S RNA. Figure 2 shows that 9S RNA is similarly cleaved by archaeal, human, and *E. coli* extracts. The major processing products correspond in size to the expected 7S, p5S, and 4S RNA species. Bacteriophage T4 gene 32 mRNA, which contains a hairpin structure downstream of the RNase E cleavage site, was also tested. Specific bands corresponding to the expected RNase E endoribonucleolytic cleavage products were detected (data not shown). The archaeal RNase E-like activity was also found to be $Mg2^+$ dependent and to generate a 5′ phosphate group, as has been shown for RNase E and some other endoribonucleases (data not shown) (4). We concluded that *Archaea* organisms contain an endoribonuclease activity that processes three well-defined bacterial RNase E substrates similarly to *E. coli* RNase E.

Partial purification of RNase E-like activity from archaea and its serological relatedness with *E. coli* RNase E. To assess the identity of the protein responsible for the RNase E-like activity from archaea, we tested the proteins in the extracts for immunological cross-reactivity with monoclonal antibodies raised against a fragment of *E. coli* RNase E. Western blots were done with total archaeal protein extracts. *E. coli* and human proteins of P-100 extracts were loaded in parallel. Three major polypeptides with molecular masses of 55, 45, and 30 kDa, respectively, were detected in the archaeal extract from *H. marismortui* (Fig. 3C). In *H. volcanii* and *H. halobium*, the 55- and 45-kDa bands were also detected (data not shown).

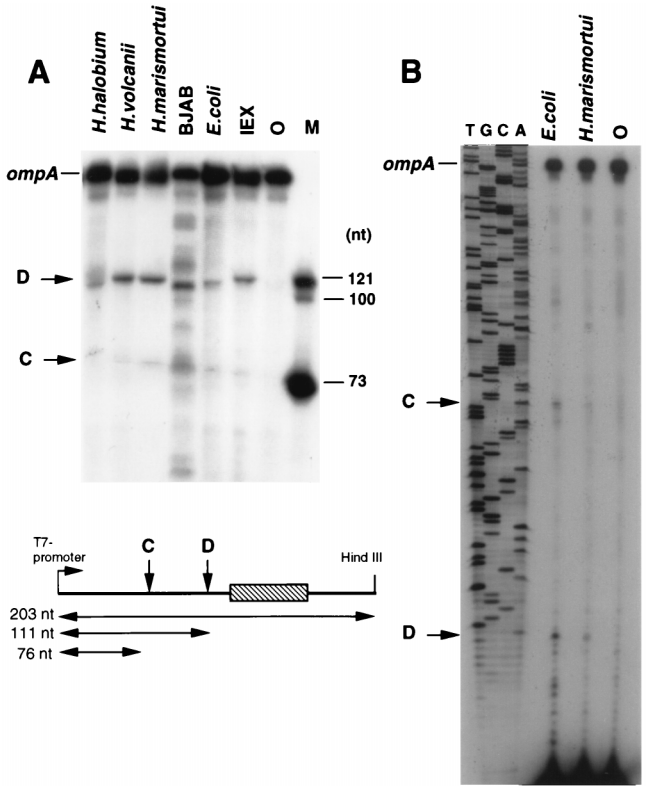

FIG. 1. In vitro RNase E activity assays with RNA transcripts from the 5′ UTR of *E. coli ompA* mRNA. RNA substrates were transcribed in vitro and incubated with different cell extracts as described in Materials and Methods. (A) The cleavage products from [α-^{32}P]UTP-labeled RNA were separated on a denaturing polyacrylamide gel and detected by autoradiography. Lanes: M, RNA molecular weight markers; O, *ompA* mRNA only without added protein; *E. coli*, *E. coli* P-100 extract; BJAB, BJAB P-100 cell extract; *H. halobium*, *H. volcanii*, and *H. marismortui*, unfractionated cytosolic extracts from archaeal cells; IEX, fraction from ion-exchange chromatography of *H. marismortui* cell extract. The positions of the full-length transcript (*ompA*) and RNase E-specific cleavage products C and D are indicated. The positions of the T7 promoter and the restriction sites are depicted at the bottom. Vertical arrows C and D mark RNase E cleavage sites relative to the template. The coding region of the *ompA* gene is represented by a hatched bar. nt, nucleotides. (B) Cleavage sites in the 5′ UTR of *ompA* mRNA. Unlabeled RNA substrate was digested with different protein extracts and then analyzed by primer extension. Lanes: O, blank in which the template was incubated in the absence of proteins. *E. coli*, digestion with a protein extract prepared from a strain overexpressing RNase E from *E. coli*; *H. marismortui*, digestion with unfractionated cytosolic extracts from archaeal cells; T, G, C, and A, DNA sequence established by dideoxy sequencing with the same oligonucleotide that was used for primer extension. The positions of the extension products corresponding to the downstream RNA fragment generated by the C and D RNase E-specific cleavages and that of the full-length *ompA* extension product are indicated.

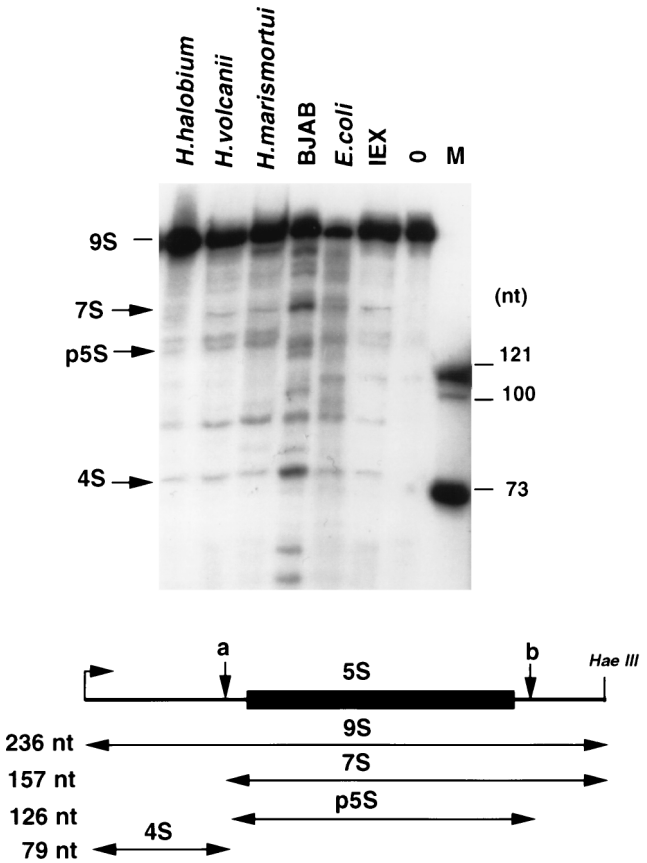

FIG. 2. In vitro processing assay with 9S RNA. Lanes M, O, *E. coli*, BJAB, *H. halobium*, *H. volcanii*, *H. marismortui*, and IEX are the same as in Fig. 1A. The positions of 9S RNA and specific maturation products are marked. At the bottom is a map of the in vitro transcripts used in the assay. Vertical arrows a and b are RNase E cleavage sites relative to the template. The 5S RNA region is represented by a solid bar. nt, nucleotides.

The immunodetection of several polypeptides that are smaller than the full-length protein is a trait common to both crude and purified RNase E preparations from *E. coli*, since the protein is very sensitive to proteolysis (6, 7, 10). Multiple bands were also seen in a Western blot analysis when cells were freshly disrupted in Laemmli sample cocktail and directly analyzed on a gel (data not shown). Figure 3C also shows that the human extract contains one polypeptide that weakly cross-reacted with the antibody. It is worth mentioning that a human RNase E-like activity has been reported to cross-react with a polyclonal antibody that recognizes bacterial RNase E (40). These data indicate that a protein immunologically related to *E. coli* RNase E also exists in *Archaea* with epitopes that are evolutionarily conserved in *Bacteria*, *Archaea*, and *Eukarya*. The cross-reactivity with the monoclonal antibodies suggests that there is a higher degree of structural conservation of the RNase E-like enzymes between *Archaea* and *Bacteria* than between *Bacteria* and *Eukarya.*

In eubacteria and eukaryotes, RNase E has been found to be associated with the polysomal fraction (37, 40). To check for subcellular localization in *Archaea*, equal fractions of polysomal (P-100) and nonpolysomal (S-100) extracts from *H. marismortui* were analyzed by Western blotting with *E. coli* RNase E monoclonal antibody G144-25.2. Figure 3C shows that in *Archaea*, the polypeptides immunologically related to RNase E are located predominantly in the nonpolysomal fraction. Soluble and polysomal extracts were assayed for RNase E-like activity by using the 5′ UTR of *ompA* mRNA as the substrate. The results presented in Fig. 3B show that the processing activity is located in the soluble nonpolysomal fraction, like the polypeptides cross-reacting with the RNase E antibody. This strongly suggests that the endoribonuclease activity in *Archaea* is due to a soluble RNase E-like enzyme.

To strengthen the link between the three immunoreactive soluble polypeptides and the RNase E-like activity from *Archaea*, proteins from an *H. marismortui* S-160 extract were fractionated by ion-exchange chromatography under halophilic conditions to avoid low-salt denaturation of the proteins. Each fraction was tested for RNase E activity under high-salt conditions and for cross-reactivity against the RNase E antibody

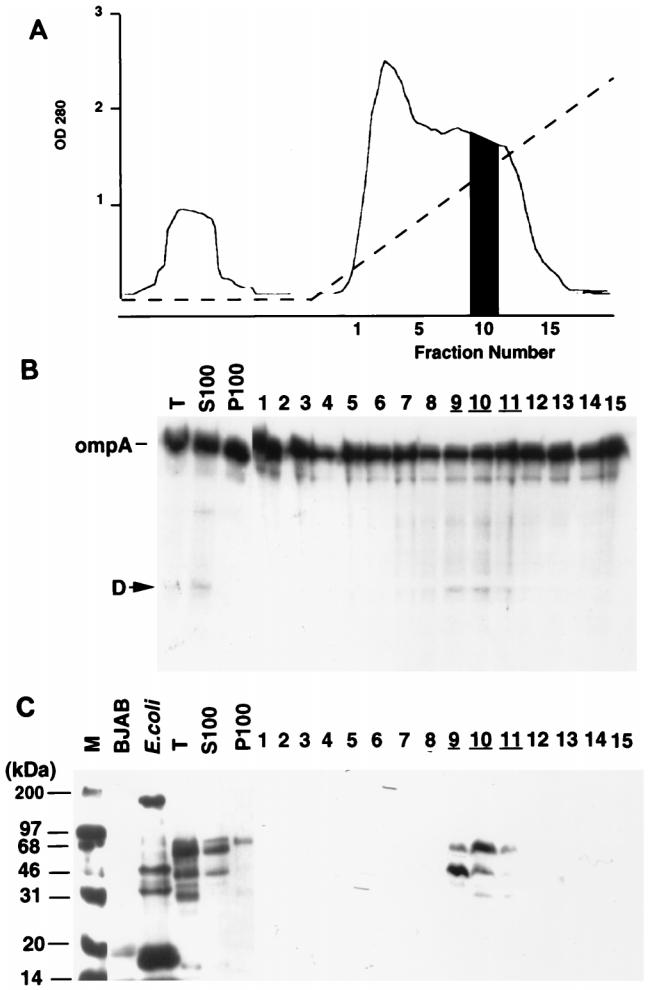

FIG. 3. (A) Partial archaeal RNase E-like activity purification. *H. marismortui* cell extracts were fractionated by ultracentrifugation and ion-exchange chromatography. The upper panel represents the A_{280} (OD 280) profile of the DE-52 chromatography. The area corresponding to the fractions containing both RNase E-like activity and the proteins cross-reacting with the RNase E antibody is darkened. (B) RNase E activity profiles. RNase E activity in fractions of the elution peak was assayed by using ^{32}P-labelled *ompA* mRNA as the substrate. The full-length transcript (*ompA*) and the main RNase E-specific cleavage product, D, are indicated. The activity was found in the soluble postribosomal supernatant and eluted in 3 of the 15 fractions (underlined). Lanes: T, total cytosolic extract; S100, postribosomal soluble fraction; P100, polysomal pellet; 1 to 15, DE-52 ion-exchange column fractions from S-160 supernatant. (C) Western blot analysis of fractionated cell extracts by using a monoclonal antibody raised against *E. coli* RNase E. Aliquots of *E. coli* and human P-100 extracts were run on a sodium dodecyl sulfate-polyacrylamide gel in parallel with fractionated extracts from *H. marismortui*. Proteins were electroblotted onto a Hybond polyvinylidene difluoride filter. The membrane was probed with a monoclonal antibody and detected with the enhanced-chemiluminescence kit from Amersham. Two main reactive polypeptides (55 and 45 kDa, respectively) were detected in all *H. marismortui* active fractions. Lanes: M, molecular weight markers; BJAB, human BJAB P-100 cell extract; *E. coli*, *E. coli* P-100 extract; T, total cytosolic extract from *H. marismortui*; S100, *H. marismortui* postribosomal soluble fraction; P100, *H. marismortui* polysomal pellet; 1 to 15, DE-52 ion-exchange column fractions from *H. marismortui* S-160 supernatant.

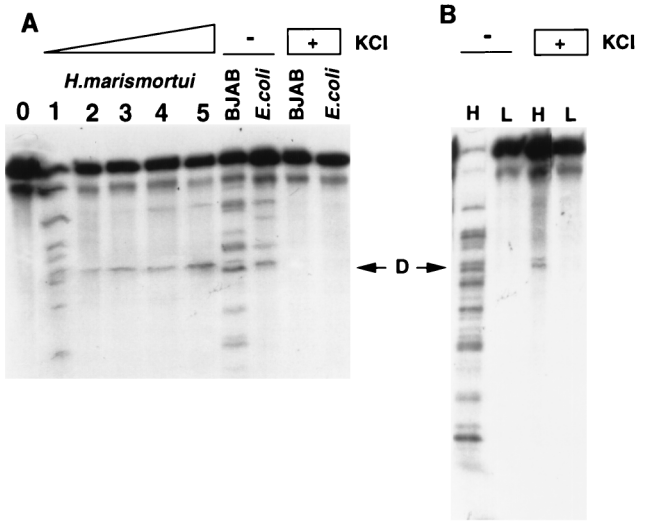

FIG. 4. (A) Salt dependence of *H. marismortui* RNase E activity. Active fractions from an ion-exchange column were incubated, as described in the text, in different salt concentrations with the 5′ UTR from *ompA* mRNA as the substrate. Human and *E. coli* P-100 fractions were assayed similarly under low-salt and extreme high-salt conditions. The main RNase E-specific cleavage product, D, is indicated. Lanes: O, *ompA* mRNA only, without added protein; 1 to 5, partially purified *H. marismortui* RNase E incubated in standard reaction buffer plus 0, 0.5, 1, 2, or 3 M KCl, respectively; *E. coli* (−) and BJAB (−), *E. coli* and human P-100 extracts incubated in low-salt standard buffer; *E. coli* (+) and BJAB (+), same extracts incubated in standard buffer plus 3 M KCl. (B) Salt dependence of *H. marismortui* RNase E stability. Ion-exchange active fractions were extensively dialyzed against low-salt or high-salt buffer and incubated further. Residual RNase E activity was measured with the 5′ UTR from *ompA* mRNA as the substrate. Lanes: H, RNase E incubated under high-salt conditions; L, RNase E incubated under low-salt conditions; −, residual activity measured under low-salt conditions; +, residual activity measured under high-salt conditions.

by Western blotting analysis. Figure 3B shows that specific RNase E cleavage product D was found only in fractions containing the three polypeptides which are immunologically related to *E. coli* RNase E. As shown by the A_{280} chromatography profile, the immunoreactive fractions represent about 10% of the total proteins loaded on the column. Furthermore, the specific activity in the RNase E-containing fractions was found to be 10-fold higher than that in S-160 extracts. We therefore concluded that RNase E-like endonuclease activity copurified with the proteins cross-reacting with RNase E. This strongly suggests that *Archaea* contains an RNase E counterpart that cleaves the same RNA substrates as RNase E from eubacteria under extremely salty conditions.

It is not possible to make a decisive statement on the size of the archaeal RNase E-like enzyme. Since *E. coli* RNase E is very susceptible to proteolysis (25), it is likely that our RNase E preparations also consisted of catalytic polypeptides smaller than the full-length archaeal RNase E protein. This is supported by the copurification of the three immunoreactive polypeptides on DE-52 chromatography, which suggests that they have closely similar physicochemical properties. It is also possible that, as in *E. coli* and mammals, the RNase E activity from *Archaea* is purified as a large heteroprotein complex, suggesting that the elution profile is determined not only by the RNase E fragments but also by the cofactors (6, 36a).

Salt dependence and stability of halophilic RNase E-like activity. Halophilic proteins require a high salt concentration to be soluble and stable (2, 13). To determine the biochemical properties of the halophilic RNase E-like activity from *H. marismortui*, we first examined the effect of increasing KCl concentrations on the enzymatic activity. Equal amounts of partially purified RNase E were incubated for 1 h with the 5′ UTR of *ompA* mRNA in different buffers (Fig. 4A). These experiments show that the specificity of the enzyme is increased by salt. If no KCl is added to the reaction buffer, the enzyme is still active but generates other, nonspecific bands.

On the contrary, RNases E from *E. coli* and human P-100 extracts are totally inhibited under high-salt conditions. To examine the stability of halophilic RNase E under different salt conditions, two samples of the partially purified activity were dialyzed, one sample against a high-salt buffer (standard processing reaction buffer plus 3 M KCl) and the other against a buffer without KCl. After 48 h of incubation, the residual processing activities were tested on the 5′ UTR of *ompA* mRNA (Fig. 4B). The results show that only the protein sample that was dialyzed against the high-salt buffer maintained processing activity under low- and high-salt conditions. We concluded that, like most of the other halophilic proteins studied, the RNase E-like enzyme requires a high salt concentration for stability. Halophilic proteins from *Archaea* are adapted to function at extreme cytosolic salt concentrations (up to 4 M KCl). In the case of the halophilic RNase E-like activity, our results suggest that, like many halophilic proteins, protein stability requires a high salt concentration. To explain this salt dependence, a model has been proposed by workers in our laboratory in which the stabilization and solvation of a halophilic enzyme are accounted for by the interaction of acidic residues on the protein surface with hydrated salt ions, showing particularly close involvement of the solvent environment in protein folding and stability (2, 13, 42). Therefore, the alteration of the processing pattern of the halophilic RNase E-like enzyme that we observed under low-salt conditions could be due to partial denaturation of the enzyme, leading to accumulation of an intermediate processing product.

Conclusion. In the present work, we have demonstrated that, like the eukaryotic RNase E-like activity, an archaeal enzyme can specifically recognize and cleave eubacterial mRNA species, showing that RNases E from all three kingdoms can process an alien piece of RNA with the same specificity. The activity was shown to have the same specificity as *E. coli* RNase E and to cross-react in a Western blot analysis with a highly specific monoclonal antibody raised against *E. coli* RNase E. From this we conclude the existence of an enzyme in *Archaea* which is structurally related to bacterial RNase E. Recently, a eukaryotic RNase E activity has also been found in human cells (40). In this context, the identification of an RNase E-like protein in *Archaea* supports the idea that enzymes involved in mRNA decay are very ancient and strongly evolutionarily conserved. This is consistent with the notion of a primitive world based on RNA and makes it likely that the origin of RNase E predates the divergence of *Archaea*, *Bacteria*, and *Eukarya.*

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