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A human RNase E-like activity that cleaves RNA sequences involved in mRNA stability control

(AUUUA motifs/mRNA decay/endoribonucleases/c-*myc*)

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ABSTRACT **We have detected an endoribonucleolytic activity in human cell extracts that processes the *Escherichia coli* 9S RNA and outer membrane protein A (*ompA*) mRNA with the same specificity as RNase E from *E. coli*. The human enzyme was partially purified by ion-exchange chromatography, and the active fractions contained a protein that was detected with antibodies shown to recognize *E. coli* RNase E. RNA containing four repeats of the destabilizing motif AUUUA and RNA from the 3′ untranslated region of human c-*myc* mRNA were also found to be cleaved by *E. coli* RNase E and its human counterpart in a fashion that may suggest a role of this activity in mammalian mRNA decay. It was also found that RNA containing more than one AUUUA motif was cleaved more efficiently than RNA with only one or a mutated motif. This finding of a eukaryotic endoribonucleolytic activity corresponding to RNase E indicates an evolutionary conservation of the components of mRNA degradation systems.**

Studies in bacteria have suggested that most of the key enzymes performing the rate-limiting steps of mRNA degradation are endoribonucleases, which recognize features of nucleotide sequence and secondary structure within the target mRNA (1). Endoribonucleolytic cleavages have also been discussed in connection with mRNA decay in mammalian cells. Recently, a cleavage that seems to initiate decay in the 3′ untranslated region (UTR) of the transferrin receptor mRNA has been reported (2). Thus, the search for cytoplasmic endoribonucleases that are involved in RNA processing is important for understanding the posttranscriptional control of mammalian gene expression.

Short-lived human oncogene and growth factor mRNAs belong to a category of mRNA in which the 3′ UTR is implicated in the control of mRNA stability. Repeats of the nucleotide sequence AUUUA have been found in this region. Sequences containing more than one of these repeats have been shown to be sufficient for conferring a short half-life when added to the 3′ UTR of otherwise stable mRNA species. Although the AUUUA sequence seems to be important for mRNA stability, it is not known whether it is the actual target for endoribonucleases initiating the rapid decay (3).

In *Escherichia coli*, three endoribonucleases have been well characterized: RNase P (4), RNase III (5), and RNase E (6). In particular, RNase E, although originally described as an rRNA processing enzyme, has been found to have a central and in many cases rate-limiting role in the control of mRNA stability in *E. coli* (for review, see ref. 7). Well-recognized targets for *E. coli* RNase E include 9S RNA, the 5′ UTR of *ompA* mRNA, and the 5′ UTR of phage T4 gene *32* mRNA (8–11). Mutants in the *rne* locus, encoding RNase E, show severe disturbances in bulk mRNA decay and become inviable at a lower temperature than wild-type strains.

Previously, eukaryotic analogues to the bacterial processing enzymes RNase P (12) and RNase III (13) have been found, demonstrating an evolutionary conservation of endoribonuclease systems. In the present study, we used human cell extracts in an *in vitro* RNA degradation assay for detecting endoribonucleases with the same specificity as RNase E. We found that extracts from all tested human cell lines contain a magnesium-dependent activity that cleaves the prokaryotic RNA substrates similarly to *E. coli* RNase E. This RNase E-like activity was further purified from polysomal pellets with ion-exchange columns. In the active fractions, a protein cross-reacting with antibodies that bind to *E. coli* RNase E was identified. The fractions with RNase E-like activity also specifically cleaved the AUUUA motif and introduced cleavages at several sites in the c-*myc* 3′ UTR. We conclude that human cells contain a strongly conserved RNase E-like activity capable of cleaving RNA sequences known to mediate rapid mRNA decay.

MATERIALS AND METHODS

Cell Lines, Bacterial Strains, Plasmids, and Oligoribonucleotides. The eukaryotic cell lines used are listed in Table 1. The human Epstein–Barr virus-negative B-cell line BJAB and the promonocyte cell line U937 were used for preparation of eukaryotic extracts for protein purification. The *E. coli* strains CAN 20-12E/18-11 (14) (RNase I−, II−, D−, BN−, T−), N3431 (15) (*rne*^{ts}), and N3433 (15) (*rne*$^{+}$) were used for preparation of bacterial extracts.

The plasmid pTH90 contains the sequence for 9S RNA and has been described (16). Plasmid pT7ompA+5* (17), a kind gift from L.-H. Chen and J. Belasco (Harvard Medical School), contains the 5′ UTR of *ompA* mRNA, a part of the coding region, and an additional inverted repeat in the 3′ end. The c-*myc* plasmid pT3mycNEpA was constructed by cloning the 370-bp *Nsi* I/*Eco*RI fragment from pSPmyc3.1 (18) [a kind gift from M. Groudine (Fred Hutchinson Cancer Research Center)] into the *Pst* I/*Eco*RI sites of pBS+. The c-*myc* fragment contains the whole 3′ UTR except the first 72 bp downstream of the stop codon in the sequence. It also includes a 59-bp poly(A) tail following the second poly(A) site in the c-*myc* 3′ UTR.

The plasmid pT7/T3-α-19AUUUA (19) was kindly provided by J. Malter (Tulane University School of Medicine).

The following oligoribonucleotides were synthesized and used in assays: AG1, 5′-CUCUAGAGGAUGCAGGUAAGCUUGGGUACCG-3′; AU1, 5′-CUCUAGAGGAUGCAUUUAAGCUUGGGUACCG-3′; AU2, 5′-CUAGAGGAUGCAUUUAUUUAAGCUUGGGUAC-3′; AU4, 5′-AGGAUGCAUUUAUUUAUUUAUUUAAGCUUGG-3′; AU1G, 5′-CUCU-

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Abbreviations: UTR, untranslated region; PNK, polynucleotide kinase.

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Table 1. RNase E activity in different cells

Cell line	Origin/type	RNase E activity
BJAB	B-cell lymphoma	+
Ramos	Burkitt lymphoma	+
Molt-4	T-cell lymphoma	+
CCRF-CEM	T-cell thymoma	+
HL-60	Promyelomonocytic lymphoma	+++
K-562	Erythroid leukemia	++
U-937	Promyelomonocytic lymphoma	+++
Saos-2	Osteosarcoma	++
MCF-7	Breast carcinoma	++
PBL	Peripheral blood leukocytes	+++
E. coli	CAN 20-12E/18-11	++

Comparison of RNase E-like activity in different eukaryotic cell lines. The levels of RNase E-like activities in different cell lines were analyzed by incubating AU4 oligoribonucleotides, which were 5′-labeled with [γ-^{32}P]ATP, in RNase assays with equal amounts of total protein from different cell lines. The activities were compared to the activity in *E. coli*. The differences in relative activities of the RNase E-like enzymes are shown, and each + indicates a difference of at least 1 order of magnitude. Other Burkitt lymphoma cell lines were also tested (i.e., P3HR-1, Namalwa, Jijoye, and DG-75) and found to contain approximately the same levels of RNase E-like activity as Ramos.

AGAGGAUGCAUUUAAGCUUGGGUACCGGGG-3′; AUMYC, 5′-CUUUAACAGAUUUGUAUUUAAGAAUUGUUUUUAAAAAAUUUUAAGAUUUACACA-3′.

The AUMYC RNA sequence was derived from the c-*myc* sequence (GenBank data base, accession no. X00364; nt 7416–7460).

Preparation of Cell Extracts and Protein Purification. P-100 extracts from *E. coli* were prepared as described (16). The BJAB and U937 cells were maintained in RPMI 1640 medium/10% fetal calf serum containing penicillin at 0.06 mg/ml and streptomycin at 0.1 μg/ml. Cells (10×10^9) were collected and washed in 0.9% NaCl. The steps for making cell extracts were performed at 4°C. After centrifugation at 4000 $\times$ *g* for 15 min, the pellet was dissolved in 4 ml of buffer A [20 mM Tris·HCl, pH 7.8/5 mM $MgCl_2$/0.1 mM EDTA/5% (vol/vol) glycerol/0.1 mM dithiothreitol]. The cells were lysed by 15 strokes of pestle B in a Dounce homogenizer and then incubated for 30 min after addition of phenylmethylsulfonyl fluoride (0.1 mM), aprotinin (2 μg/ml), leupeptin (1 μg/ml), pepstatin A (1 μg/ml), and 10 μg of DNase I (DPRF; Worthington). P-100 extraction, ammonium sulfate precipitation, and fractionation on a Mono Q column (Pharmacia) were performed as described (16). The fractions were assayed for RNase E activity. Aliquots of the active fractions were precipitated with 12% trichloroacetic acid, centrifuged, washed with cold acetone, and air dried. The samples were then dissolved in SDS sample mixture and loaded on a SDS/15% polyacrylamide gel (20). After electrophoresis, the gel was blotted onto Optitran BA-S 83 (Schleicher & Schuell) in a buffer containing 25 mM Tris·HCl, 192 mM glycine, and 20% MeOH. The membrane was blocked with 5% nonfat dry milk (Maresi, Vienna) and probed with a rabbit polyclonal antiserum (1:10,000) (kindly provided by B. Holland, University of Paris-Sud) made against yeast heavy chain myosin and shown to react with *E. coli* RNase E (21). Horseradish peroxidase-conjugated anti-rabbit IgG (1:5000) was used as the second antibody and detected with the ECL detection system from Amersham.

Unfractionated eukaryotic cytoplasmic extracts were prepared as described (22).

***In Vitro* Transcription, RNase E Activity Assay, and RNA Sequencing.** RNA substrates were transcribed from 2 μg of linearized plasmids in the presence of [α-^{32}P]CTP essentially as described (10). pTH90 and pT7ompA+5* were linearized with *Hae* III and *Hin*dIII, respectively. pT7/T3-α-19AUUUA and pT3mycNEpA were linearized with *Eco*RI. The synthesized RNA oligomers were labeled at the 5′ end with [γ-^{32}P]ATP and T4 polynucleotide kinase (PNK) (Boehringer Mannheim) according to the manufacturer's instructions. RNase E activity was assayed as described (10). For RNA sequencing, unlabeled 9S RNA was used in parallel with labeled RNA samples for RNase activity assays and run on denaturing polyacrylamide gels. The unlabeled RNA bands corresponding to the p5S and 7S RNA products were excised from the gel, eluted, and dephosphorylated with calf intestine alkaline phosphatase (Boehringer Mannheim). The RNAs were 5′-labeled using PNK and [γ-^{32}P]ATP; subjected to partial RNase digestion with the enzymes RNase T1, RNase U2, and RNase PhyM (USB); and run on a 20% sequencing gel.

To determine whether the RNase cleavages give a 5′-phosphate group, assays with unlabeled 9S RNA and *ompA* mRNA were performed. The reaction mixture was split and half was dephosphorylated with calf intestine alkaline phosphatase while the other half was untreated. Both halves were then phosphorylated with [γ-^{32}P]ATP and PNK. The reaction mixtures were precipitated and run on a 6% denaturing polyacrylamide gel. Fuji RX x-ray film was used for all autoradiography.

RESULTS

RNase E Substrates Are Processed in the Same Manner in Both Prokaryotic and Eukaryotic Extracts. The rRNA processing intermediate 9S from *E. coli* was used to assay for RNase E-like activity in cellular extracts. The different rRNA species in *E. coli* are produced from a large single RNA precursor by RNA processing, and the 9S RNA intermediate is further processed by RNase E cleavage at two sites, leading to the formation of p5S RNA. When *in vitro* transcribed ^{32}P-labeled 9S RNA substrate was incubated with extracts from *E. coli* or from the human B-lymphoma cell line BJAB, similar processing products appeared (Fig. 1*A*). They correspond in size to the expected 7S, p5S, and 4S RNA species (16). The human RNase activity was recovered when polysomal

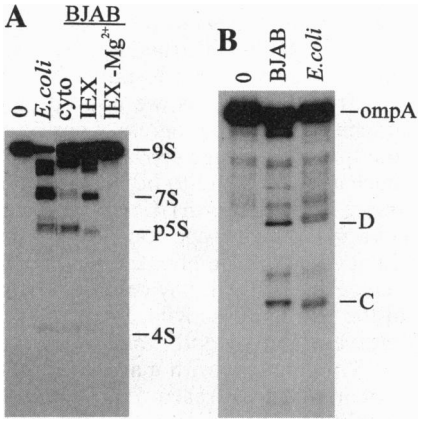

FIG. 1. *In vitro* RNase E activity assays with RNA substrates from 9S RNA and the 5′ UTR of *ompA* mRNA. *In vitro* transcribed RNA substrates were incubated with different cellular extracts as described. The cleavage products were analyzed on denaturing polyacrylamide gels. (*A*) Assay with 9S RNA. Lanes: 0, 9S RNA only, without added protein; *E. coli*, *E. coli* P-100 extract; cyto, unfractionated cytoplasmic extract from BJAB cells; IEX, fraction from ion-exchange chromatography of BJAB cell extract; IEX-Mg^{2+}, same as IEX but without magnesium. Positions of 9S RNA and cleavage products are indicated on the right. (*B*) Assay with the 5′ UTR of *ompA*. Lanes: 0, *ompA* mRNA only; BJAB, unfractionated cytoplasmic extract from BJAB cells; *E. coli*, *E. coli* P-100 extract. The full length transcript (*ompA*) and the RNase E-specific cleavage products [C and D (9, 10)] are indicated on the right.

proteins from BJAB cells were fractionated by ion-exchange chromatography (Fig. 1*A*, lane 4). The last lane in Fig. 1*A* is a control showing that the human RNase activity, like that of *E. coli*, is magnesium dependent. To investigate the capability of the human RNase activity to recognize other known prokaryotic RNase E targets, it was assayed with the 5′ UTR of the *E. coli ompA* mRNA. As in the assays with 9S RNA, the expected processing products C and D (16) appeared after incubation with prokaryotic as well as eukaryotic extracts (Fig. 1*B*).

To verify that the p5S and 7S RNA products are identical, unlabeled RNA cleavage products were eluted from a gel and 5′ labeled. The resulting, purified RNA molecules were subjected to partial digestion with base-specific RNases and analyzed on a sequencing gel. The p5S and 7S products were found to have identical internal RNA sequences and 5′ ends (data not shown). Furthermore, when unlabeled 9S RNA and *ompA* mRNA were used in RNase assays, and then either treated or not treated with alkaline phosphatase before labeling with [γ-^{32}P]ATP using PNK, RNA that had been phosphatase treated was preferentially labeled (data not shown). This was found for both prokaryotic and eukaryotic extracts and indicates that the processing products have 5′-phosphate groups. It should be noted that in *E. coli* the processing of RNA by RNases E, H, P, and III generates 5′-phosphate groups, while RNases I, I*, M, and R yield 5′-hydroxyl groups (23).

Additional bands apart from the expected ones are present in Fig. 1. These could be due to minor differences in substrate preference between the human and prokaryotic RNase activities, or the preparations may contain a modifying factor(s) or another RNase. However, previous studies have shown that highly purified *E. coli* RNase E gives rise to more than the expected cleavage products (24, 25). We conclude that human BJAB cell extracts contain a magnesium-dependent, polysome-associated activity that processes two well-defined RNA substrates similarly to *E. coli* RNase E. The human activity is therefore an RNase E-like enzyme. Table 1 shows that the activity could be detected in cell lines of various origin, in peripheral blood leukocytes, and at levels that differ by ≈2 orders of magnitude.

Partial Purification and Immunological Cross-Reactivity with RNase E Antibodies. Prompted by the finding that an RNase E-like enzyme is conserved from bacteria to humans, we investigated the possibility of structural conservation of the enzyme. When we originally tried to purify the activity processing 9S RNA from BJAB cells, we noticed that a 65-kDa protein was enriched during the progress of purification. In this preparation, the specific cleavage activity in the fractions with the endoribonuclease was found to be ≈100-fold higher than in the cell extract (data not shown). Later, we recognized that U937 cells had a much higher specific cleavage activity than BJAB cells (Table 1), and we applied extracts from these cells to an ion-exchange chromatography column for further characterization of the enzymatic activity. Fractions were assayed for 9S RNA cleavage activity and probed with the RNase E antibodies (21). The fractions with maximal cleavage activity contained a protein that cross-reacted with the antibody (Fig. 2). The size of this protein was again 65 kDa, which makes it significantly larger than the previously reported human 13-kDa Ard protein, which has been shown to complement RNase E deficiency in *E. coli* and to produce RNase E-like cleavages (26). However, we could not detect a protein with a size comparable to the full-length *E. coli* RNase E [180 kDa on SDS/PAGE (21, 24, 25)].

RNase E Cleaves the AUUUA Sequence Motif. Considering the known preference of *E. coli* RNase E for target sequences rich in A and U residues (7), we tested the AUUUA sequence motif that has been implicated in the control of mRNA decay for certain short-lived human mRNAs (27–29). The pentanucleotide AUUUA has been suggested to constitute a functional sequence determinant for mRNA stability, but

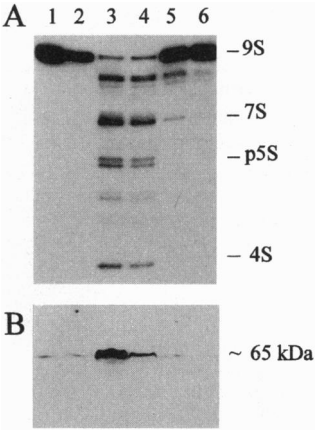

FIG. 2. (*A*) RNase E activity profile of the Mono Q column. P-100 extract from 10 × 10^9 U937 cells was separated by ion-exchange chromatography in a Mono Q column. Fractions (lanes 1–6) were assayed for RNase E activity, using 9S RNA as substrate. Positions of cleavage products are indicated on the right. Main activity was found to elute at a KCl concentration of 0.2–0.3 M. (*B*) Western blot of the Mono Q fractions. Aliquots from the same fractions were run on a SDS/polyacrylamide gel and then electroblotted onto a nitrocellulose filter. The membrane was probed with antibodies reactive against *E. coli* RNase E (21) and detected with the ECL kit from Amersham.

apparently more than one repeat of this element is necessary to influence mRNA half-life (3). There is a proportionality between the number of AUUUA repeats and the reduction in mRNA stability, which has been shown in studies where the AUUUA motifs were introduced in the 3′ UTR of an otherwise stable β-globin mRNA (3, 28). A construct with four repeats of AUUUA (19) was therefore used initially to assess the capability of the human RNase E-like enzyme to recognize such motifs.

When using RNA from this construct, a ladder-like pattern of degradation products appeared, due to cleavages within the AUUUA repeat (Fig. 3*A*). The cleavage products were analyzed on a sequencing gel (data not shown), revealing that the cleavages occurred exactly after the second U residue in each AUUUA motif. To demonstrate that RNase E mediated these cleavages, extracts from the *E. coli rne*^{ts} strain N3431 were incubated with the RNA. As shown in Fig. 3*A*, a reduction of cleavages was found when the mutant extracts were incubated at the nonpermissive temperature (45°C; lane 3). Reduced cleavage at 45°C was not seen when extracts isolated from the wild-type strain N3433 were used (data not shown). These findings show that the AUUUA repeats are targets for both the bacterial RNase E and its human counterpart.

Qualitative and Quantitative Correlation Between Number of AUUUA Repeats and RNase E Cleavages. Bearing in mind the correlation between the number of AUUUA motifs and the extent of half-life reduction *in vivo*, we investigated whether such a correlation could be found *in vitro*. Smaller RNA oligonucleotides containing one, two, four, or no AUUUA motifs were synthesized and used in degradation assays. Fig. 3*B* shows that the human RNase E-like enzyme cleaved these substrates once, twice, four times, and not at all, respectively. Thus, the AUUUA motif is a recognition site for the human RNase E-like enzyme. Whether the number of sites also gives rise to faster decay kinetics was addressed by incubating substrates containing one or four AUUUA motifs in the same assay reaction mixture and measuring their half-lives *in vitro*. Fig. 3*C* shows that the RNA with four AUUUA repeats decayed considerably faster than the RNA containing only one copy of AUUUA. Densitometric evaluation of the autoradiogram gave an approximate half-life difference of a factor of 4–5 between the two RNA substrates. Thus, *in vitro*,

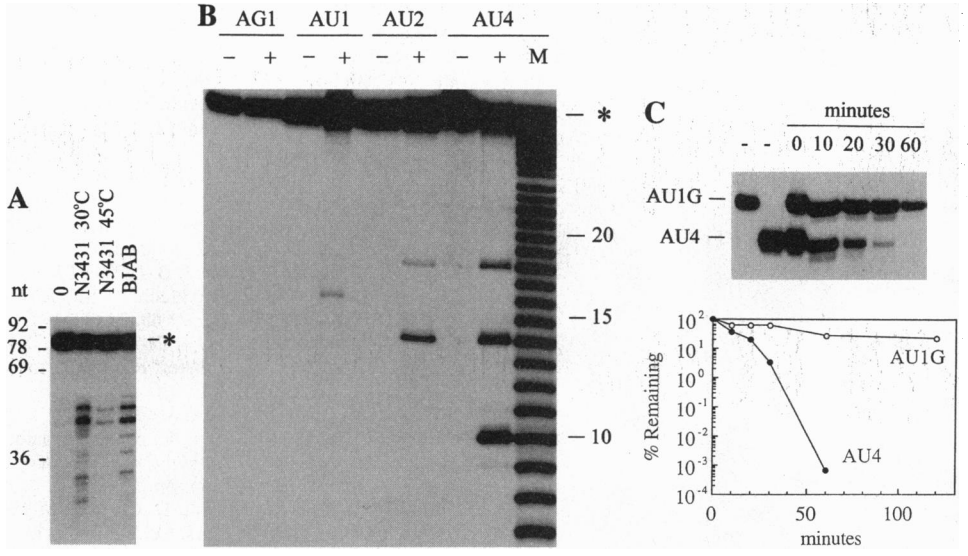

FIG. 3. *In vitro* RNase degradation of AUUUA motifs. (*A*) *In vitro* transcribed 80-nt RNA from pT7/T3-α-19AUUUA (19) was incubated with P-100 extract from the RNase E^{ts} mutant *E. coli* strain N3431 at 30°C and at 45°C and with unfractionated extract from BJAB cells. The size of the full-length transcript is marked with an asterisk. Lane 0, RNA only. Sizes are shown on the left. In the sequence 5′-GGGAAAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUCCAUU·UAUU·UAUU·UAUU·UAAGCUUGGGUACCGAGCUCGAAUU-3′, the A+U-rich region is underlined and the cleavage sites (·) are marked. (*B*) *In vitro* degradation assays with different oligoribonucleotides. Oligonucleotides were labeled with [γ-^{32}P]ATP and incubated with ion-exchange-purified BJAB P-100 extracts. Lanes: −, RNA only; +, RNA incubated with extract; M, alkaline hydrolysis ladder of the AU4 oligomer. Full-length bands (*; 31 nt) and sizes of the ladder are shown on the right. AG1 (...AGGUA...) is not cleaved, whereas AU1 (...AUU·UA...), AU2 (...AUU·UAUU·UA...), and AU4 (...AUU·UAUU·UAUU·UAUU·UA...) were cleaved at the indicated positions (·). (*C*) Kinetic comparison of cleavage efficiency for the oligoribonucleotides AU4 and AU1G. AU1G is AU1 with 3 additional G residues in the 3′ end to make it distinguishable from AU4 in length. Equal amounts of the labeled AU1G and AU4 were incubated with ion-exchange-purified BJAB P-100 extract for various times. Lanes −, labeled RNA without protein extract added. Graph shows a decay plot with the remaining RNA vs. time. The AU4 RNA was found to decay ≈4.5 times faster than the AU1G RNA.

the half-life decreases in proportion to the number of AUUUA recognition sites, as has been found to be the case *in vivo* (3).

The Human RNase E-like Enzyme Cleaves the c-*myc* mRNA 3′ UTR at Several Sites. Although the 3′ UTR of many short-lived mRNA species contains multiple repeats of the AUUUA motif, this is not invariant. c-*myc* mRNA has dispersed AUUUA motifs, which does not necessarily explain its relatively short half-life, since isolated AUUUA motifs also occur in the 3′ UTR of stable mRNAs—e.g., β-globin (30). The natural 3′ UTR of c-*myc* mRNA was therefore assayed for other possible RNase E target sites. When probing this RNA with the human RNase E-like enzyme, several degradation intermediates were observed (Fig. 4*A*). A region from the middle of the UTR was found to contain a number of putative RNase E sites [A/G-AUU-A/U (7)]. A synthetic RNA oligomer covering this region was synthesized and found to be cleaved at 7 sites (Fig. 4*B*). The cleavages occurred at positions that resemble common RNase E cleavage sites, but only two of them are located within AUUUA motifs. This result confirms that a natural human 3′ UTR mRNA can be cleaved by the human RNase E-like enzyme and that the target sites do not correspond to a single sequence motif.

DISCUSSION

We have described an endoribonuclease activity from human cells that cleaves bacterial and mammalian RNA substrates with the same specificity as RNase E from *E. coli*. Fractions harboring the endoribonuclease showed reactivity with an antiserum against bacterial RNase E. Substrate specificity, cross-reactivity with RNase E antibodies, and magnesium dependence strongly suggest that the identified endoribonuclease is a mammalian RNase E-like enzyme.

At this point, it is difficult to make a decisive statement on the size of the identified enzyme. It is known from RNase E preparations from *E. coli* that the enzyme is labile and vulnerable to proteolytic degradation (7). Preparations with the human RNase E-like enzyme have been obtained in which the catalytic polypeptide was definitely smaller than the previously determined size of the full-length RNase E [180 kDa on SDS/PAGE (21, 24, 25)]. The 65-kDa protein reacts on Western blots with antibodies that also detect *E. coli* RNase E; a similar-sized protein was purified from BJAB cells. The immunological cross-reactivity indicates a structural conservation similar to that found in human and prokaryotic RNase P enzymes (31). The identification of mammalian proteins that can reverse mRNA decay deficiency in *E. coli* (26), the cross-reactivity of anti-myosin antibody with RNase E (21), and the involvement of the hsp60 homologue GroEL in mRNA decay (9, 16) indicate a conservation of several components of mRNA degradation systems between pro- and eukaryotes.

We have found that bacterial and human RNase E activities recognize, as cleavage substrates, RNA sequences known to confer a reduced half-life in human cells when engineered into the 3′ UTR of otherwise relatively long-lived mRNA species. Endoribonucleolytic cleavages in these regions have so far not been described *in vivo* (i.e., in steady-state total cellular RNA), but such degradation intermediates are likely to be extremely short lived and present as a minute fraction of the full-length mRNA species. In *E. coli*, the primary products of RNase E cleavage have been detected for certain mRNA species. These primary products were found to be identical to those produced in the *in vitro* assay used in the present study (10).

Our present *in vitro* data on the partially purified human RNase E-like enzyme show that AUUUA is a recognition site for this enzyme. Furthermore, our *in vitro* data demonstrate a correlation between the number of these recognition sites and the rate of RNA decay. These findings may explain why repeated AUUUA motifs reduce RNA half-life *in vivo*. We note that a recent study suggests the minimal half-life-determining consensus sequence to be UUAUUUAUU (32), which corresponds to a partial AUUUA triplet.

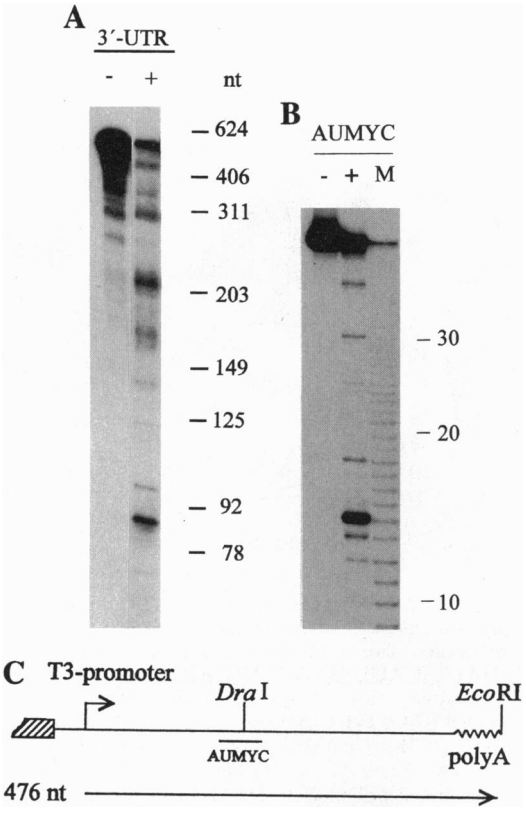

FIG. 4. *In vitro* RNase E assays with the 3′ UTR of c-*myc* mRNA and a shorter c-*myc* oligoribonucleotide. (*A*) Degradation assay of the 3′ UTR from c-*myc*. *In vitro* transcribed RNA from pT3mycNEpA was incubated in the presence (lane +) or absence (lane −) of ion-exchange-purified BJAB P-100 extract. (*B*) Degradation of the oligoribonucleotide AUMYC. The 54-mer AUMYC was labeled in the 5′ end with [γ-^{32}P]ATP and incubated with ion-exchange-purified BJAB P-100 extract. Lanes: −, RNA only; +, RNA incubated with the BJAB cell extract. Cleavages (·) were found at several sites inside the RNA: 5′-CUUUAACAGAUU·U·G·UAUU·UAAGAAUUGUUU U·UAAAAAAUUU·UAAGAUU·UACACA-3′. (*C*) *In vitro* transcripts for c-*myc* derived from plasmid pT3mycNEpA. Positions of the T3 RNA polymerase initiation sites and the restriction sites for making run-off transcripts are shown. Part of the coding region is indicated by a hatched bar. AUMYC shows location of the oligoribonucleotide used for mapping cleavages within the A+U-rich sequence, and poly(A) shows the location of the 59-nt poly(A) tail.

The 3′ UTR of c-*myc* has been identified as one region that is responsible for the relatively short half-life of this transcript (33). Although this region is A+U-rich, it does not contain the typical repeats of the AUUUA motif, which we identify as one motif that makes transcripts susceptible to RNase E attacks. If RNase E cleavage activity is involved in the short half-life of the c-*myc* mRNA, one would expect the 3′ UTR to exhibit clusters of cleavage sites not identical to the AUUUA motifs. Our demonstration of multiple cleavage sites in the 3′ UTR of c-*myc* mRNA indicates that the AUUUA motif may be only a subset of RNase E cleavage sites.

Since the AUUUA motif is also the target for RNA-binding proteins (19, 34), it may have a dual function, serving as a cleavage site and a binding site. Our identification and partial purification of an endoribonuclease capable of introducing specific cleavages in these RNA sequence motifs may serve as a foundation for further exploration of this model.

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