The CafA Protein Required for the 5'-Maturation of 16 S rRNA Is a 5'-End-dependent Ribonuclease That Has Context-dependent Broad Sequence Specificity*

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The CafA protein, which was initially described as having a role in either Escherichia coli cell division or chromosomal segregation, has recently been shown to be required for the maturation of the 5'-end of 16 S rRNA. The sequence of CafA is similar to that of the N-terminal ribonucleolytic half of RNase E, an essential E. coli enzyme that has a central role in the processing of rRNA and the decay of mRNA and RNAI, the antisense regulator of CoIE1-type plasmids. We show here that a highly purified preparation of CafA is sufficient in vitro for RNA cutting. We detected CafA cleavage of RNAI and a structured region from the 5'-untranslated region of ompA mRNA within segments cleavable by RNaseE, but not CafA cleavage of 9 S RNA at its "a" RNase E site. The latter is consistent with the finding that the generation of 5 S rRNA from its 9 S precursor can be blocked by inactivation of RNase E in cells that are wild type for CafA. Interestingly, however, a decanucleotide corresponding in sequence to the a site of 9 S RNA was cut efficiently indicating that cleavage by CafA is regulated by the context of sites within structured RNAs. Consistent with this notion is our finding that although 23 S rRNA is stable in vivo, a segment from this RNA is cut efficiently by CafA at multiple sites in vitro. We also show that, like RNase E cleavage, the efficiency of cleavage by CafA is dependent on the presence of a monophosphate group on the 5'-end of the RNA. This finding raises the possibility that the context dependence of cleavage by CafA may be due at least in part to the separation of a cleavable sequence from the 5'-end of an RNA. Comparison of the sites surrounding points of CafA cleavage suggests that this enzyme has broad sequence specificity. Together with the knowledge that CafA can cut RNAI and ompA mRNA in vitro within segments whose cleavage in vivo initiates the decay of these RNAs, this finding suggests that CafA may contribute at some point during the decay of many RNAs in E. coli.

The overproduction of CafA (1–3) under conditions of slow growth has been shown to cause the formation of chained cells and minicells. The presence of the latter has been interpreted as evidence for CafA either enhancing the rate of cell division and/or inhibiting chromosome partitioning after replication (4). Electron microscopic examination of the chained cells revealed axial filamentous bundles, termed cytoplasmic axial filaments (hence the designation CafA), running through the center of their cytoplasmic filaments. Furthermore, the cytoplasmic axial filaments appear to be composed almost entirely of CafA (5). This finding combined with the phenotype of cells overproducing CafA has led to the proposal that in normal cells these filaments in an unbulded form may have a role as cytoskeletal-like elements in either cell division or chromosome segregation (4).

The sequence of CafA has 34% similarity with the N-terminal nucleolytic domain of RNase E (6), an essential Escherichia coli ribonuclease that is required for the generation of 5 S rRNA from a 9 S precursor (7) and has a central role in the decay and/or processing of a variety of RNAs, including many if not most mRNAs and RNAI, the antisense RNA regulator of the replication of CoIE1-type plasmids (for reviews see Refs. 8 and 9). Endoribonucleolytic cleavage by RNase E occurs within single-stranded A and/or U-rich segments (10, 11); however, there is no simple relationship between the order of nucleotides and the phosphodiester bond(s) that is cleaved (12, 13). An oligonucleotide corresponding in sequence to the 5'-end of RNAI has been found to be cut efficiently by RNase E in vitro (14). Combined with the knowledge that alteration of secondary structures adjacent to RNAI sites can either increase or decrease the rate of cleavage (11, 14, 15), this finding has contributed to the notion that secondary structures within complex RNAs rather than serving as direct recognition motifs (14, 15) affect RNase E cleavage by either limiting access of the enzyme (14, 16, 17) and/or determining the stability of local structures, which in turn determines the single-strandedness (and thus cleavability) of susceptible sites (15, 18, 19).

Recently, it has been shown that efficient cleavage by RNase E is dependent on the nature of the 5'-end of its substrates. Compared with a linear substrate that had a 5'-monophosphate group immediately followed by a single-stranded segment, circular substrates, 5'-triphosphorylated substrates, or 5'-monophosphorylated substrates that had a duplex at the extreme 5'-end were found to be cut inefficiently by RNase E in vitro (20). The two latter observations are consistent with the in vivo findings that pppRNAI was decayed more slowly than pppRNAI in vitro (21) and 5'-stem-loops (or rather the absence of an unpaired segment) can stabilize RNA (16, 22), respectively. Additionally, the preferential cleavage by RNase E of 5'-monophosphorylated RNAs, such as downstream fragments produced by this enzyme (e.g. pRNAI), over 5'-triphosphorylated intact RNAs (e.g. pppRNAI) suggests that once decay of an RNA molecule is initiated its completion will be preferred over the cutting of intact RNA (20). This notion may explain the observation that in general RNAs decay without the accumulation of significant levels of decay intermediates, the so called...
“all or nothing” phenomenon (20).

RNase E has recently been shown to be also capable of removing 3’-poly(A) tails (23), which are known to facilitate 3’-exonucleolytic attack by polynucleotide phosphorylase (PN-Pase; for reviews see Refs. 24–27). Furthermore, it was reported that the poly(A) nuclease activity of RNase E is blocked by the presence of a 3’-monophosphate group suggesting that it is 3’-exonucleolytic. Although, the precise role of the poly(A) nuclease activity of RNase E in RNA decay remains to be determined, it seems likely that any action it has on poly(A) tails in vivo would affect processing by PN-Pase, which together with RNase E, the RhlB helicase, and the glycolytic enzyme decay.

To investigate whether it has ribonucleolytic activity. Here we purified CafA and investigated whether it has ribonucleolytic activity. We report that CafA is indeed a ribonuclease, which is consistent with the recent finding that it is required for the 5’-maturation of 16S ribosomal RNA (37, 38). Furthermore, the results of our investigation into the nature of the ribonucleolytic activity of CafA raise the possibility that it may also have a role in RNA decay.

EXPERIMENTAL PROCEDURES

Chemical Synthesis of RNA Oligonucleotides—Oligoribonucleotides BR10, BR10p, and A40 were synthesized using an ABI 391 DNA synthesizer with a modified 1-μmol DNA assembly cycle and standard ABI reagents, and the coupling efficiencies were determined by trityl assay as described previously (39). Deprotected RNAs were purified by anion exchange chromatography using a Dionex DNA Pac PA-100 column (4 × 250 mm) on a Dionex DX500 high pressure liquid chromatography unit.

In Vitro Transcription—RNA, 9 S RNA, and the 5’-UTR of ompA mRNA were synthesized using the T7-MEGAshortscript from Ambion. Typically 50 nm of DNA template was incubated at 37 °C for 90 min in a 20-μl reaction as described by the vendor. When internally labeled RNA was required, 60 μCi of [α-32P]UTP (ICN) was included in the reaction. Transcripts were visualized by either UV shadowing or autoradiography (40) and gel purified. 9 S RNA and a segment of the 5’-UTR of ompA mRNA were generated from HaeIII-cut pTH90 (41) and HindIII-cut p106B-64 (a gift from J.G. Belasco, Skirball Institute), respectively, whereas the template for the synthesis of RNAI was a polymerase chain reaction product generated using primers with sequences

5′-AACCGATATAAGGGATATTGGTG and 5′-AACAAAAACCGTACACGAGCCAG. The ribonucleolytic activities of CafA were determined by incubating with 20 μl of 20 μM Tris-HCl (pH 7.5), 3.3 mM dithiothreitol, 10 mM MgCl₂, 10% (v/v) Me₂SO, 10 units of T4 RNA ligase (Amersham Pharmacia Biotech), and 33 pmol (100 μCi) of [5′-32P]polytide 3′,5′-bisphosphosphate (ICN). After incubation at 4 °C for 2 h, the reactions were quenched with urea-loading buffer, and the labeled RNAs were gel purified.

Results—Labeled full-length RNA was separated from unincorporated forms and unincorporated nucleotides by running in polyacrylamide sequencing gels. 8 and 20% (v/v) polyacrylamide gels were used to purify transcripts and oligoribonucleotides, respectively. An autoradiograph of the gel was used as a template, and a slice of gel containing the radiolabeled RNA was excised. RNA was eluted from the gel slice into 400 μl of 150 mM NaCl, 50% (v/v) acetylated phenol (pH 4.3) at 65 °C for 2–16 h. The eluate was extracted with phenol and chloroform, precipitated by ethanol, and resuspended in water (Sigma).

Construction of pCAFA01 Encoding His-tagged CafA—The cafA gene segment was amplified from plasmid pMEI1 (42) using an Elongase kit as per the vendor’s instructions (Life Technologies, Inc.). The primers used were 5′-GGCCGCGCATATGACGCTGAATTGTTAGTAAAGCG and 5′-GCCGGAATCTTACATCATCCATGACTGTCACATGTCG, which introduced unique NdeI and BamHI sites (bold type) at the 5′- and 3′-end, respectively, of the cafA coding sequence. The resulting 1.4-kilobase polymerase chain reaction product was cut with NdeI and BamHI and cloned between the corresponding sites of PET11b (Novagen). The resulting plasmid was designated pCAFA01.

Purification of CafA and the N-terminal Catalytic Domain of RNase E—(E. coli BL21(DE3)) cells harboring either pCAFA01 (this work) or pNSTOP (31), a plasmid encoding the N-terminal ribonucleolytic domain (residues 1–498) of RNase E, were grown in 2YT (40) to an A₅₀₀ of 0.6. Expression of the plasmid-coded gene was then induced by adding isopropyl-1-thio-β-D-galactopyranosidase to 1 mM. After induction for a further 60 min at 37 °C, the cells were harvested by centrifugation at 4000 × g for 10 min using a MSE 3000 centrifuge. The cell pellet was resuspended in 50 ml of binding buffer (7 mM urea, 20 mM Tris-HCl (pH 7.6), 500 mM NaCl, 5 mM imidazole, 0.1% (v/v) Triton X-100), and the cells were ruptured by passage through an French Pressure Cell (Amicon). The insoluble material including intact cells was removed by high speed centrifugation (100,000 × g, 30 min) using a Beckman SW28 rotor. The supernatant was loaded on to a 5-ml nickel-charged HiTrap Chelating column (Amersham Pharmacia Biotech), which was washed with 30 ml of binding buffer before bound proteins were eluted with an imidazole gradient (5 mM to 1 M over 20 ml). All of the above purification steps were done using buffers, columns, and equipment that have been cooled to below 4 °C. The protein content of each fraction was assayed using a modified Bradford assay (Bio-Rad) and SDS-polyacrylamide gel electrophoresis. In initial experiments (Fig. 1), CafA was purified from extracts of cells from a 1-liter culture; however, we found that purification from a cell extract of a 4-liter culture resulted in reduced levels of contaminating polypeptides (Fig. 2).

Electroelution of Purified CafA Protein—Recombinant CafA protein was further purified from a preparative Lamelli gel (40) using a Mini Whole Gel Eluter (Bio-Rad). Protein was eluted from a 10% (w/v) polyacrylamide gel (7.2 × 10 cm, 0.1 cm) as per the vendor’s instructions into 50 mM Tris-HCl (pH 8.7), 25 mM boric acid. A voltage of 200 V (10 watt) was applied for 30 min. At the end, the polarity was reversed for 15 s to remove any protein that might have stuck to the membrane during elution. Residual SDS was removed from the eluate samples using Exracti-gel D as per the vendor’s instructions (Pierce).

RNA Cleavage Reactions—Two hundred ng of either CafA or RNase E, purified as described for 4–15 μl of the appropriate substrate RNA in 20 μl of 20 μM Tris-HCl (pH 7.4), 100 mM NaCl, 10 mM MgCl₂, 0.1% (v/v) Triton X-100, and 1 mM dithiothreitol containing 20 units of RNase inhibitor (Amersham Pharmacia Biotech) at 37 °C for up to 60 min. Samples were taken at regular intervals and quenched using urea-loading buffer before aliquots were analyzed using polyacrylamide sequencing-gels (gels 40).

RESULTS

CafA (RNase G) Is a 5′-End-dependent” Endoribonuclease—Fractions across a peak of recombinant His-tagged CafA that was purified using immobilized metal affinity chromatography (Fig. 1A) were incubated with 5′-labeled BR10 (5′-ACAGUAUUUG), a synthetic decarboxyribonucleotide that corresponds in sequence to the single-stranded segment at the 5′-end of RNAl (14). Ribonuclease activity was detected in all fractions containing CafA (B); moreover, the level of activity was directly proportional to the amount of this polypeptide (C).

1 The abbreviations used are: UTR, untranslated region; nt, nucleotides(s).
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To confirm that CafA was the source of the ribonucleolytic activity, we further purified CafA by electroeluting, following preparative gel electrophoresis, a batch that contained reduced amounts of contaminating polypeptides (compare Fig. 1A and Fig. 2A) as a result of increasing the amount of cell extract added to the immobilized metal affinity chromatography column (see "Experimental Procedures"). The resulting CafA preparation (Fig. 2) was homogeneous as judged by staining of SDS-polyacrylamide gels using Coomassie Blue (A) and silver (data not shown) and was still able to cleave the decanucleotide substrate (B). Thus, from here on we will adopt the designation RNase G (37, 38) when referring to CafA. Comparison of the migration of the upstream products of RNase G cleavage with a 1-nt ladder generated using PNPase revealed that they were 5, 6, and 7 nt (B).

To determine whether the products of RNase G cleavage were generated endoribonucleolytically, we used as substrate 5'-monophosphorylated BR10 labeled at its 3'-end by the addition of [5'32P]pCp. As shown in Fig. 3, downstream products of 4, 5, and 6 nt were detected (A) consistent with endonucleolytic cleavage at the same positions identified using 5'-labeled BR10. Moreover, the relative abundance of each of the downstream products (Fig. 3A) mirrored precisely that of the corresponding upstream products (Fig. 2) indicating that RNase G is only able to cut individual BR10 decanucleotides once. Incubation of RNase G with two BR10 derivatives that had either three extra Gs at the 5'-end or a C at the 3'-end resulted in cleavage at precisely the same positions (relative to sequence) observed for BR10 (data not shown), indicating that as found for RNase E (14), the specificity of RNase G cleavage of BR10 is determined by sequence rather than a distance measured in nucleotides from either its 3'- or 5'-end.

We next investigated whether efficient RNase G cleavage is dependent on the presence of a monophosphate on the 5'-end of its substrates by incubating with RNase G an aliquot of 3'-labeled BR10 that had not been 5'-phosphorylated (Fig. 3B). We were unable to detect cleavage of this substrate by RNase G after 60 min of incubation (B) even though 50% of 5'-monophosphorylated BR10 was cut within 2 min (A). This finding indicates that like RNase E, which was included as a control, RNase G is a 5'-end-dependent ribonuclease (20). In contrast, we found that the 3'-phosphorylation status did not affect the rate of cleavage of 5'-labeled BR10 by RNase G or RNase E; an oligonucleotide synthesized with a 3'-phosphate was cleaved as efficiently as one that had a hydroxyl group at its 3'-end (Fig. 4).

The Specificity of RNase G Cutting Overlaps That of RNase E and Is Context-dependent—Having found that RNase G can cut BR10 (Fig. 3), we decided to investigate using complex substrates whether RNase G can cut within other single-stranded segments cleavable by RNase E and/or would cut at other positions. To this end, we incubated RNase G and, as a control, RNase E with RNAI, the 5'-UTR of ompA mRNA, 9 S RNA, and a segment from 23 S rRNA (Fig. 5). All of the substrates used were labeled at their 5'-ends. Our RNase E preparation cut RNAI primarily at the −5 position within the single-stranded region at its 5'-end. Less efficient RNase E cleavage at the −6
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Fig. 3. Assay of ribonucleolytic cleavage of substrates mono-
phosphorylated or hydroxylated at their 5'-ends. A, 3'-Labeled
BR10 that had been monophosphorylated at its 5'-end was incubated
with RNase G (CafA) and, as a control, RNase E (NRne, 50 ng) as
described in Fig. 1. The RNA species marked with an asterisk is 5'-
hydroxylated BR10 resulting from incomplete 5'-phosphorylation. B
was the same as A, except the substrate had not been 5'-monophos-
phorylated using T4 polynucleotide kinase (see “Experimental Proce-
dures”). The double asterisk indicates a minor contaminating species
generated during gel purification.

Fig. 4. Assay of ribonucleolytic cleavage of 5'-labeled sub-
strates having either a monophosphate or hydroxyl group at
their 5'-ends. 5'-Labeled BR10 (5'-32P) was incubated with RNase G
(CafA) and, as a control, RNase E (NRne, 50 ng) as described in Fig. 1.
The sample was analyzed in the gel described in “Experimental Proce-
dures”. The single asterisk indicates RNA that had its 3'-phosphate removed by the
3'-phosphatase activity of T4 polynucleotide kinase during the 5'-label-
ing reaction (see “Experimental Procedures”), whereas the double as-
terisks show the position of a contaminating species generated during
preparation of the substrate. The latter provides a convenient internal
control for loading differences.

position and at internal sites within RNAI was also evident as
observed previously (14, 36). RNase G was also able to cut
RNAI; moreover, the major sites of cutting overlapped those of
RNase E within the single-stranded segment at the 5'-end (A).
Relative to RNase E, however, RNase G was able to cut more
efficiently at the -6 position but appeared to be unable to
cleave at internal sites. In the case of the 5'-UTR segment of
ompA mRNA, we found that RNase G was able to cut within
two internal segments that contain major RNase E sites des-
ignated “c” and “d” (43, 44), albeit more slowly than our RNase E
preparation (B). Close examination of this gel suggests that
although RNase G is able to cut within the c segment of ompA
mRNA, the precise bonds that were cleaved differ from those
cut by RNase E. We were unable to detect RNase G cleavage at
the a RNase E site of 9 S RNA (C), which is required for the
generation of 5 S rRNA (45), indicating that not all RNase E-
cleavable segments within complex RNAs are substrates for
RNase G. This finding is consistent with the observation that 9
S RNA processing can be blocked in cells that are wild type for
RNase G (46). Taken together these results indicate that al-
though RNase E and RNase G specificity overlap, they are not
congruent.

We further examined the basis of the inability of RNase G to
cleave within the a site of 9 S RNA by incubating it with a
synthetic decanucleotide that corresponds in sequence to this
segment of RNA. As shown in Fig. 6, RNase G was able to
cleave this substrate as efficiently as RNase E indicating that
the context of the a site in 9 S RNA and not its sequence blocks
cleavage by RNase G. Consistent with the context being im-
portant in regulating RNase G cleavage of sites within complex
RNAs is our finding that although 23 S rRNA is stable in vivo,
A segment of this RNA was cleaved efficiently and at multiple
sites by RNase G (Fig. 5D).

RNase G Has Only Weak Poly(A) Nuclease Activity—As
RNase E has recently been shown to be able to remove 3'-
poly(A) tails (23) in addition to making decay-initiating cleav-
gs at or near the 5'-end of RNAs (for reviews see Ref. 8), we
also incubated RNase G with a mixture of equimolar amounts of a 5'-labeled A40-mer and BR10 (Fig. 7). The N-terminal half
of RNase E cut the A40-mer at a rate that was reproducibly
3–4-fold slower than that of BR10; however, even though
RNase G under identical reaction conditions cleaved 50% of
BR10 within 6 min, it only cut 25–30% of the A40-mer after 90
min (A). Similar rates of decay were obtained when the sub-
strates were incubated individually with RNase G or RNase E
(B). Combined these results indicate that relative to RNase E,
RNase G has weak poly(A) nuclease activity.

DISCUSSION

The work presented here extends recent reports that RNase
G is required for the maturation of the 5'-end of 16 S rRNA in
vivo (37, 38). We have shown that a highly purified preparation
of RNase G is sufficient in vitro for endonucleolytic cutting of
RNA (Figs. 1–3). Moreover, we find that efficient cutting by this
enzyme, like that by RNase E, is 5'-dependent (20); under
the experimental conditions used we were unable to detect
RNase G (or RNase E) cleavage of a 5'-hydroxylated oligoribo-
nucleotide that was cleaved efficiently when monophosphory-
lated at its 5'-end (Fig. 3). We also found that RNase G cleav-
age was blocked by the presence on substrates of a 5'-
triphosphate group (data not shown), as reported for RNase E
(20). Therefore, we suggest that, as proposed for RNase E (20),
RNase G will prefer to cut to completion 5'-monophosphory-
lated decay or processing intermediates rather than initiating
the decay of intact 5'-triphosphorylated RNAs. Implicit in this
model is that 5'-monophosphoryl groups only stimulate cleav-
age at sites present on the same RNA molecule. Support for
this is provided by our finding that 5'-hydroxylated RNA pres-
ent at a low level in our preparation of 3'-labeled 5'-monophos-
phorylated BR10 (as a result of incomplete enzymatic 5'-phos-
phorylation; see “Experimental Procedures”) was not cleaved
by RNase G or our RNase E preparation even after the bulk of
the 5'-monophosphorylated RNA had been cut to completion
(Fig. 3A).
As the RNase E polypeptide we used as a control contained only the N-terminal ribonucleolytic domain and homologues of this half of RNase E have been found in the genomes of all Gram-negative and some Gram-positive bacteria that have been completely sequenced (32), we suggest that the mechanism for sensing the presence of monophosphate groups on the 5'-end of RNAs may be ancient and evolutionarily conserved. It is also conceivable that the distance separating a cleavable sequence(s) from a 5'-monophosphate group may control the efficiency of cutting by RNase G and RNase E. Indeed this notion, which is open to experimental investigation, may provide an explanation for the observation by others that RNase G is only able to generate the mature 5'-end of 16 S rRNA in vivo after cutting of 17 S RNA by RNase E (38), and our finding that although RNase G is unable to cut 5'-monophosphorylated 9 S RNA at the a RNase E site (Fig. 5); it is able to cut as efficiently as the N-terminal domain of RNase E a decanucleotide corresponding in sequence to this segment of RNA (Fig. 6). In both these examples a 5'-monophosphate group is brought closer (at least in terms of sequence length) to a segment that can be cleaved by RNase G. An additional possibility, which need not be mutually exclusive, is that higher order structures within 17 and 9 S RNA block access of RNase G to potentially cleavable sequences. Indeed, there is good evidence that the overall conformation of RNA can affect the efficiency of cutting by RNase E (10, 11, 16–19). In any case, the context of a sequence within a complex RNA, such as 17 S RNA, is likely to be extremely important (and possibly the major factor) in determining whether it will be cleaved by RNase G, as examination of the sequences cleaved by this enzyme did not reveal a requirement for a particular order of nucleotides (Fig. 8). Similar conclusions have been reached regarding the specificity of RNase E cleavage (12, 13).

Our finding that RNase G can cleave in vitro at multiple positions within the 5'-single-stranded sequence of RNAI (Figs. 2a and 5A) raises the possibility that it also contributes to the decay of this RNA in vivo. Consistent with this notion is the finding that when products of cleavage in the 5'-single-stranded region of RNAI were stabilized in vivo by mutational inactivation of either the gene encoding PNPase or the polymerase that adds poly(A) tails to RNAI, species generated by cleavage at the –6 and –7 positions were detected in addition to RNase E cleavage at the –5 position (47, 48). Additionally, when RNase E was mutationally inactivated in vivo, RNAI (from both pACYC184 and pBR322) was still decayed, albeit more slowly, and certain cleavages within its 5'-end appeared

Fig. 5. Assay of ribonucleolytic cutting of RNAI, the 5'-UTR of ompA mRNA, 9 S RNA, and a segment from 23 S rRNA. Each 5’-labeled substrate was incubated with RNase G (CafA) and RNase E (NRne) as described in Fig. 1. RNAI, the 5'-UTR of ompA mRNA and 9 S RNA in A–C, respectively, were transcribed in vitro, whereas the segment of 23 S rRNA (residues 1052–1075; Ref. 56) in D was synthesized chemically (57). The single asterisk in A indicates a minor species generated by cleavage of an RNA transcript(s) that is 1–2 nt shorter at the 5'-end than full-length RNAI. In C, the minor species marked with a double asterisk in the preparation of 9 S RNA substrate result from cleavage during gel elution (see “Experimental Procedures”). In D, lanes 1, 2, 3, and 4 contain samples incubated with no enzyme, RNaseG, RNase E, and PNAase, respectively. The latter incubation generated a 1 nt-ladder that was used to determine the sizes of the endonucleolytic cleavage products.

Fig. 6. Assay of ribonucleolytic cutting of an RNA segment containing the a RNase E of 9 S RNA. 5’-Labeled 9SA, a decanucleotide (5’-ACAGAAUUG) that corresponds in sequence to the a site of 9 S RNA, was incubated with RNase G (CafA) and RNase E (NRne) as described in Fig. 1. Samples of the reactions were run in a 20% (w/v) polyacrylamide sequencing gel. The sizes of the products were determined by comparing their migration against that of a 1 nt-ladder of the substrate generated using PNPase (data not shown).
be unaffected (12, 13).

The enzymatic activity that cleaves within the 5'-UTR of ompA mRNA was originally designated RNase K as several lines of evidence at that time suggested it was distinct from that of RNase E (43). Contributing to this conclusion was the finding that an activity that cleaved the 5'-UTR of ompA mRNA could be chromatographically separated from an activity of RNase E, i.e. the generation of pre-5 S rRNA from a 9 S precursor (for review see Ref. 49). Later, it was shown that a proteolysed preparation of RNase E could cut a site within the 5'-UTR of ompA, and it was proposed that RNase K activity was due to a fragment of RNase E (44, 50). However, this notion was not sufficient to explain why the fractions that cleaved the 5'-UTR of ompA mRNA did not cleave 9 S RNA and were not inactivated by the heating of extracts from a temperature-sensitive RNase E strain of E. coli (49). In light of our results (Fig. 5), an alternative explanation is that the 5'-UTR of ompA mRNA was cleaved by RNase G in these experiments (38). Furthermore, a role for RNase G in the cleavage of the 5'-UTR of ompA mRNA in vivo would provide a mechanism to explain how the decay of ompA mRNA and the processing of 9 S RNA can be differentially regulated (51). In any case, the finding that RNase G can make cleavages that resemble those of RNase E (this work, 37, 38) combined with evidence that the functions of RNase E and RNase G overlap genetically, albeit only partially (52), suggests that the study of decay events controlled by endoribonucleolytic cleavages now requires a careful assessment of the effect of mutational inactivation of RNase G in addition to that of RNase E. For example, it is possible that RNase G contributes to the processing of the mRNA of key cell division genes, as has been found for RNase E (53, 54), thus providing an explanation for the finding that overproduction of RNase G disrupts normal cell division (4).

Fig. 7. Assay of ribonucleolytic cutting of a poly(A) segment. A, an equimolar mixture of 5'-labeled A40 (a polymer of 40 As) and BR10 was incubated with RNase G and RNase E, and samples that were taken at different time intervals (given at the top of panel) were run in a 10% (w/v) polyacrylamide gel. Double asterisks are used as described in Fig. 4. B, plot of the percentage of substrate remaining with time. Squares and triangles represent the amount of BR10 remaining in RNase G and RNase E reactions, respectively. Similarly, circles and diamonds represent the amount of A40 remaining in RNase G and RNase E reactions, respectively. Closed symbols represent measurements taken from reactions that contain a mixture of both substrates (A), whereas open symbols represent measurements taken from reactions (not shown) that contained only a single substrate. The values were derived from phosphoimages as described in Fig. 1.

The biological role, if any, of the cytoplasmic axial filaments formed by RNase G (4) remains to be determined; however, we note with interest that there is a precedent in eukaryotes for associations between RNA and cytoskeletal filaments that have key roles in fundamental cellular processes (for review see Ref. 55). Regardless, the ability of RNase G to self-associate in vivo may explain our finding (data not shown) that this enzyme aggregates in vitro at high concentrations (>1 mg/ml) necessitating its purification and storage under denaturing conditions.

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