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RNase E-Dependent Cleavages in the 5′ and 3′ Regions of the *Escherichia coli unc* mRNA

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The endonucleolytic processing of the *unc* mRNA encoding the eight subunits of the *Escherichia coli* F_1F_0-ATPase was studied. Northern (RNA) blots of mRNA expressed from a plasmid which contained the 3′-terminal portion of the operon including the *uncDC* sequences revealed, in addition to the expected 2-kb mRNA, a 0.5-kb RNA species which hybridized to an *uncC* antisense RNA probe. An *uncD* antisense RNA probe hybridized to only the 2-kb mRNA, implying that the upstream 1.5-kb fragment is rapidly degraded. The 5′ end of the 0.5-kb fragment was determined by primer extension analysis to be 11 bases into the coding region of the *uncC* gene. In RNase E-deficient strains, the amount of the 0.5-kb product was strongly reduced while the levels of the precursor *uncDC* transcript remained high. Similar RNase E-dependent processing was found in the chromosomally encoded *unc* mRNA. As this RNase E-dependent cleavage directly inactivates *uncC* and appears to leave *uncD* susceptible to degradation, it seems unlikely to play a role in differential expression of the gene products but may be an important event in *unc* mRNA degradation. RNase E mutants also showed altered processing of the chromosomally encoded *unc* mRNA in the *uncB* region near the 5′ end. The expected full-length (7-kb) transcript was recognized when RNA from the RNase E-deficient strain was subjected to Northern blot analysis with *uncB*- and *uncC*-specific probes. RNA from strains with functional RNase E lacked the 7-kb transcript but had a 6.2-kb mRNA detectable with the *uncC* but not the *uncB* probe. RNase E is therefore implicated in multiple cleavages of the *unc* mRNA.

The 7-kb *unc* operon of *Escherichia coli* contains nine cistrons, eight of which encode F_1F_0-ATPase, a membrane-bound complex which couples the translocation of protons across the membrane to ATP synthesis in the process of oxidative phosphorylation. The order of the genes has been established as *uncIBEFHAGDC* (17), and the operon has been sequenced (41). *uncI*, the first gene in the operon, encodes a small polypeptide whose function is not known. The next three genes, *uncBEF*, encode the three subunits of the membrane-embedded F_0 sector, namely *a*, *c*, and *b*, respectively. The last five genes encode δ, α, γ, β, and ε, the subunits of the F_1 sector which has the catalytic site for the ATP synthesis and hydrolysis. The stoichiometry of these subunits in the mature ATPase complex is $\alpha_3\beta_3\gamma\delta\varepsilon ab_2c_{10}$ (13).

Previous studies have shown that the subunits are produced in the appropriate relative amounts from a single polycistronic mRNA (7, 24). Any differential expression must therefore occur at the posttranscriptional level. The organization of the *unc* operon seems to bear no relationship to their rate of expression. Rather, the genes generally alternate in order between those that are expressed more and less efficiently, and they are grouped functionally.

Several studies have implied that differential intrinsic efficiencies of translation initiation among the *unc* genes provide a major contribution to the disparity in the levels of synthesis (24, 26, 27). Another potential mechanism of posttranscriptional regulation is the processing and differential degradation of mRNA. This mechanism has been demonstrated to function in regulating the differential gene expression of *E. coli* operons such as *pap* (4) and *mal* (33), but the generality of this mechanism is not yet established. We are investigating the relationship between *unc* mRNA processing and differential expression of the *unc* cistrons.

Most *E. coli* mRNAs are short lived (10), and their turnover is carried out by exonucleases and endonucleases (5). RNase II and polynucleotide phosphorylase have been shown to be the two major enzymes involved in the exonucleolytic degradation of *E. coli* mRNA in a 3′-to-5′ direction (10), but the involvement of particular endonucleases is less well understood. The *ams* (for altered mRNA stability) gene was identified by Kuwano et al. (20) as a locus required for efficient degradation of mRNA. Recently, both Taraseviciene et al. (39) and Babitzke and Kushner (3) have shown the *ams* locus to be identical to *rne*, which encodes RNase E (1). This enzyme had previously been characterized by its role in the maturation of 5S rRNA (30). More recently, RNase E-dependent endonucleolytic cleavage of several specific messengers has been characterized (see, e.g., references 31, 32, and 34). The properties that define sites that are highly susceptible to RNase E attack have yet to be completely elucidated, but the enzyme usually cleaves in A+U-rich regions (34).

In this paper we report evidence of RNase E-dependent processing in the 5′ and 3′ regions of the *unc* mRNA and discuss its implications on the overall degradation of the *unc* mRNA and on the differential expression of some of the *unc* genes.

MATERIALS AND METHODS

Bacterial strains. The *E. coli* strains used in this study are listed in Table 1.

Plasmid construction. Recombinant DNA procedures were carried out by standard methods (23). Expression plasmids bearing the *uncC* gene, constructed by using the vector pKK223-3 (6), have been described previously (11). Plasmid pSD13, which carries the 3′ end of *uncG*, all of *uncDC*, and

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TABLE 1. Bacterial strains

Strain	Genotype	Reference
JM103	*Δlac-pro* F′ *traD36 proAB lacI^{q}ZΔM15*	29
SK5003	*thr leu pnp-7 rnb-500*	10
SK5004	*thr leu pnp-7*	10
SK5005	*thr leu rnb-500*	10
SK5006	*thr leu*	10
SK5665	*thyA715 ams-1*(Ts)[a]	2, 3
MG1693	*thyA715*	2, 3
N3431	*lacZ43 thi-1 relA1 spoT1 rne-3071*(Ts)	14
N3433	*lacZ43 thi-1 relA1 spoT1*	14
1100	*bglR thi-1 rel-1* HfrP01	19
DK8	1100 Δ(*uncB-uncC*)*ilv*::Tn*10*	19

[a] Babitzke and Kushner (3) have proposed renaming the *ams-1* allele as *rne-1*.

the *unc* transcription terminator, was constructed by inserting the 2.1-kb fragment produced by partial digestion of pAP55 (8) with *Pst*I into the *Pst*I site of pUC8 (40) and selecting a clone bearing a plasmid with the insert in the proper orientation for expression. pHN1 was constructed by transferring the insert from pSD13 into pEX1 (35) by using the *Hin*dIII and *Sma*I sites. pHN6 was constructed by transferring the insert from pSD38 (11) into pTZ18U (28) by using *Eco*RI and *Hin*dIII sites. The plasmids pHN3, pHN7, and pHN8 carried the inserts described below in the direction opposite to that for expression, producing the antisense RNA to be used as *uncC*-, *uncD*-, and *uncB*-specific probes, respectively. pHN3 contained the 0.4-kb *Mnl*I-*Hpa*II fragment of the *uncC* gene cloned into the vector pTZ19U (28), which bears a promoter for T7 RNA polymerase. pHN7 was constructed by inserting the 0.25-kb *Eco*RI-*Sal*I (early *uncD* coding region) fragment of pSD13 into pTZ19U which had been cut with *Sal*I and *Eco*RI. pHN8 carried the 0.2-kb *Bam*HI-*Rsa*I (early *uncB* coding region) fragment of *uncB* gene cloned into pTZ18U. All constructs were verified by restriction endonuclease mapping.

Bacterial growth. Cells were grown at 37°C with shaking in Luria-Bertani broth (23). Selective media for the strains carrying expression plasmids contained 40 μg of carbenicillin per ml. During early logarithmic growth isopropyl-β-D-thiogalactoside (IPTG) was added to a concentration of 1 mM to induce expression. After 10 min of induction, samples were removed and RNA was extracted by the method of Mackie (22).

Temperature shift experiments. The temperature-sensitive RNase E mutant strains and their respective wild-type isogenic strains carrying pHN1 were grown at 30°C to early log phase (A_{600} = 0.2). Half of the culture was then shifted to 43°C, and the other half was kept at 30°C. After incubation for 30 min at the two temperatures, expression was induced by addition of IPTG to 1 mM. RNA was extracted from samples taken after 10 min of induction to compare steady-state pHN1 mRNA levels. Rifampin was then added to a final concentration of 0.1 mg/ml, and RNA was extracted from samples taken at various times.

Northern analysis. Samples of the extracted RNA were run on either polyacrylamide or agarose gels. In polyacrylamide gel Northern (RNA) blot analysis, RNA was size fractionated for 60 min at 150 V on a 6% polyacrylamide gel containing 8 M urea in 1× Tris-borate (TBE) buffer (23). The RNA was then electrophoretically transferred onto Zeta-probe membrane by using 0.5× TBE buffer for 60 min at 80 V in a Bio-Rad Mini Trans-Blot Cell. Agarose Northern blot analysis was performed by fractionation of RNA samples on a 1.25% agarose gel in 10 mM sodium phosphate buffer (pH 7.0) for 3 h at 80 V after treatment of RNA with glyoxal at 55°C for 60 min in the presence of dimethyl sulfoxide. The RNA was then vacuum blotted for 2.5 h to an Amersham Hybond-N filter with 3 M NaCl–0.3 M sodium citrate (20× SSC) as the transfer buffer. RNA was fixed to the membrane by illumination of the blot with UV light for 3 min and drying in vacuo for 60 min at 80°C. Prehybridization and hybridization with the appropriate probe were carried out at 55°C as described by Mackie (21).

Probes were detected by two different methods. Filters hybridized with ^{32}P-labeled probes were subjected to autoradiography for 16 to 24 h. Alternatively, hybridization was detected by using the digoxygenin-UTP (DIG-UTP) system (Boehringer Mannheim Biochemica). Filters hybridized with probes labeled with DIG-UTP were blocked with 2% "Blocking reagent for nucleic acid hybridization and detection" (Boehringer Mannheim) at room temperature for 3 h and then incubated for 30 min with high-affinity anti-DIG Fab fragments conjugated to alkaline phosphatase. The membrane was washed in 100 mM Tris-HCl–150 mM NaCl (pH 7.0) at room temperature for 1 h, with two changes of wash solution. The membrane was then incubated with the Lumi-Phos 530 substrate for 30 min at 37°C and exposed to X-ray film for 1 to 30 min to record the chemiluminescent signal.

Labeling of hybridization probes. For Northern analysis, antisense RNA transcribed in vitro by using T7 RNA polymerase was used as a probe. The *uncC* probe was prepared by the in vitro transcription of pHN3 linearized with *Nco*I, producing antisense RNA to the region between *Hpa*II and *Nco*I sites in the *uncC* gene. pHN7 linearized with *Eco*RI and pHN8 linearized with *Bam*HI were used to prepare the *uncD* and *uncB* probes, respectively. The *uncD* probe was directed to the region between the *Sal*I and *Eco*RI sites in the *uncD* gene, and the *uncB* probe was directed to the region between the *Bam*HI and *Rsa*I sites in the *uncB* gene. In vitro transcription was carried out by using 2 μg of the appropriate linearized template with 40 U of T7 RNA polymerase at 37°C for 60 min in the presence of 1 mM each ATP, CTP, and GTP and either 100 μM [α-^{32}P]UTP or 0.7 mM DIG-UTP. The reaction buffer contained 40 mM Tris-HCl (pH 8.0), 6 mM $MgCl_2$, 10 mM dithiothreitol, and 2 mM spermidine.

5′-End mapping of the processed transcript by primer extension. A synthetic oligonucleotide primer, 5′-TTAATG GCGGTGAGCAGC-3′, complementary to a region 122 to 139 bases into the *uncC* coding sequence was radioactively end labeled with [γ-^{32}P]ATP by using T4 polynucleotide kinase as described by Maniatis et al. (23). RNA was hybridized to the labeled primer by treatment for 2 min at 90°C, 10 min at 40°C, and 10 min on ice, and then the primer was extended for 45 min at 50°C by 200 U of Moloney murine leukemia virus reverse transcriptase (New England Biolabs) in the presence of 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 10 mM dithiothreitol, 3 mM $MgCl_2$ and 0.5 mM each dATP, dCTP, dGTP, and dTTP. Products were denatured and analyzed on a 6% polyacrylamide–8 M urea sequencing gel, along with the pSD38-based sequencing ladder.

RESULTS

Processing the 3′ end of the *unc* mRNA derived from various *uncC* expression plasmids. The portions of the *unc* operon carried by plasmids used in expression studies and

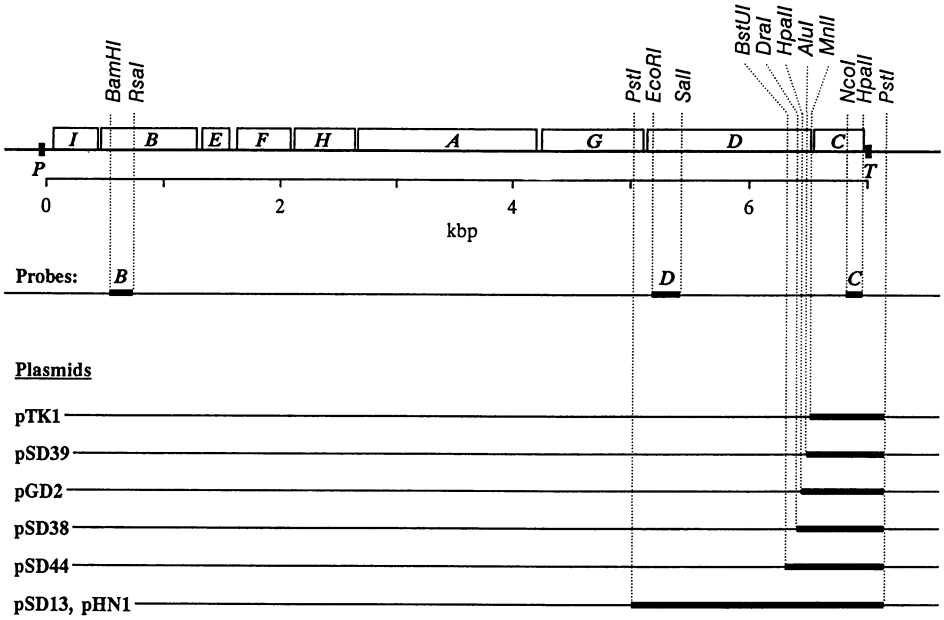

FIG. 1. The *unc* operon, segments of the operon cloned in expression plasmids or used as antisense RNA probes, and the restriction endonuclease sites defining the ends of these segments are shown. Abbreviations: *P*, promoter; *T*, terminator.

the locations of the antisense probes are shown in Fig. 1. Plasmids pSD44, pSD38, pGD2, pSD39, and pTK1 constitute a nested set of *uncC* expression plasmids containing inserted sequences beginning at various restriction sites in the *uncD* gene and extending through the *uncC* gene and the *unc* transcription terminator, as previously described (11). RNA from induced cultures of strains carrying these plasmids was analyzed by Northern blots by using an antisense RNA probe complementary to the 3′ one-third of the *uncC* sequence (Fig. 2A). The controls, an induced culture carrying pKK223-3 (lane 2) and a noninduced culture carrying pSD39 (lane 1), gave no signal. All the induced strains (lanes 3 to 7) carrying recombinant plasmids contained two species that hybridized with the *uncC* probe. Both of these species appeared only after induction, excluding the possibility that they arose from different promoters. The upper band in each case was of the expected size for the full-length mRNA; one can see that as the size of the cloned fragment increased, the size of the upper band also increased. The smaller species of mRNA, about 0.5 kb, was the same size in all the induced strains. This band was probably the 3′ end of each full-length mRNA, since all the constructs had a common 3′ end and the probe was directed toward the 3′ end of the mRNA. This suggested that the smaller species arose from the full-length mRNAs by some process of nucleolytic decay. One can estimate from the size of the smaller band that its 5′ end is near the beginning of the *uncC* gene, which encodes the ε subunit of the F_1-ATPase. The amount of upstream *uncD* sequence appeared to have little or no effect on the efficiency of the processing.

Processing of mRNA derived from a plasmid containing *uncDC*. mRNA transcribed from pSD13 (Fig. 1), which carries the entire *uncDC* region, was analyzed to determine the fate of the sequence upstream from the processing site and to determine the role of *uncD* translation on the processing event. RNA samples from a strain carrying pSD13 were analyzed by Northern blots with either *uncD*- or *uncC*-specific antisense RNA probes (Fig. 2B). The *uncC* probe recognized both the 2-kb full-length mRNA and the expected 0.5-kb processed mRNA, but the *uncD* probe recognized only the 2-kb mRNA (compare lanes 1). One might expect the *uncD* probe to recognize a 1.5-kb species, corresponding to the mRNA upstream of the processing site. The failure to detect such a product implies that, upon processing, the sequences upstream of the cleavage site were degraded quickly.

To investigate whether the translation of the 3′ end of the *uncD* mRNA just upstream from *uncC* has any effect on the processing, a stop codon was introduced by site-directed mutagenesis (9) 129 bases upstream of the normal *uncD* stop codon. The sequence of the mutagenic oligonucleotide was 5′-AGGGAGACTTATTTACCC-3′, with the underlined T providing the mismatch. This mutation changed the intercistronic region between the *uncD* and the *uncC* genes from 20 to 149 bases and shortened the β subunit, encoded by *uncD*, by 43 amino acids. Comparison of the steady-state levels of mRNA from a strain carrying the wild-type *uncDC* and the mutated *uncDC* showed no difference in the levels of the full-length and the processed mRNAs (data not shown). Thus, it seems that the translation of the 3′ region of the *uncD* mRNA has no effect on the processing.

Processing in the chromosomally encoded *unc* mRNA. To determine whether the observed processing also takes place on the chromosomally encoded *unc* mRNA, total RNA from various strains without any expression plasmid was also analyzed by Northern blots (Fig. 3). Because the chromosomally encoded mRNAs are far less abundant than those from induced multicopy plasmids, much larger amounts of RNA were used in this experiment than in the experiments above. RNA from an *unc* deletion strain, DK8 (lanes 2), was included in the experiment to distinguish *unc* transcripts from nonspecific signals. The *uncC* probe exhibited considerable affinity for the abundant rRNA species at 2.9 and 1.5 kb, while the *uncD* probes faintly recognized only the 2.9-kb species. RNA from strain 1100 (lanes 1), the unc^+ parent of DK8, contained a major *unc*-specific species of about 6 kb

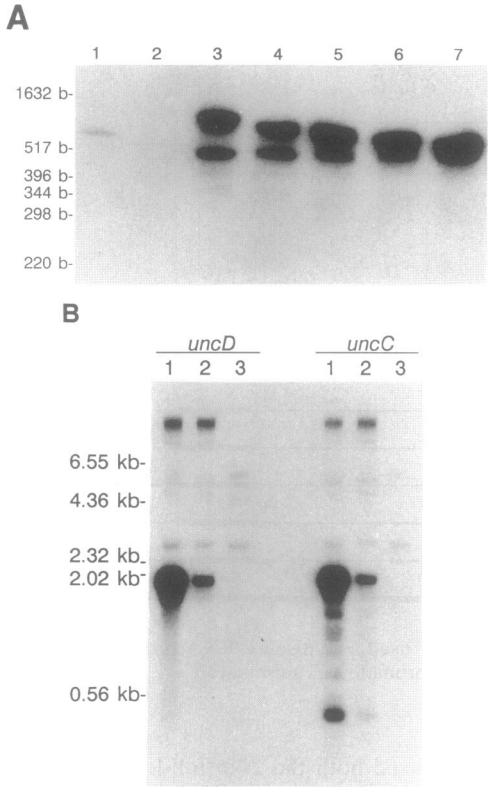

FIG. 2. Analysis of *unc* mRNA transcribed from various plasmids. (A) Northern blot analysis of mRNA transcribed from a set of *uncC* expression plasmids. Equal amounts of total RNA (1 μg) from induced *E. coli* JM103 carrying various plasmids was size fractionated on a 6% polyacrylamide–8 M urea gel, and Northern blot analyses were performed by using the ^{32}P-labeled *uncC* probe as described in Materials and Methods. RNA was obtained from *E. coli* strains bearing recombinant plasmid pSD39 (lane 1), pKK223-3 (lane 2), pSD44 (lane 3), pSD38 (lane 4), pGD2 (lane 5), pSD39 (lane 6), or pTK1 (lane 7). All samples except that in lane 1 were from induced cultures. The size markers are shown on the left (b, bases). (B) Northern blot analysis of mRNA transcribed from plasmid pSD13. RNA (1 μg) from strain JM103 bearing plasmid pSD13 (induced) (lanes 1), pSD13 (noninduced) (lanes 2), and pUC8 (induced) (lanes 3), was separated on a 1.25% agarose gel, and Northern hybridization with the ^{32}P-labeled *uncD* and *uncC* probe was carried out as described in Materials and Methods.

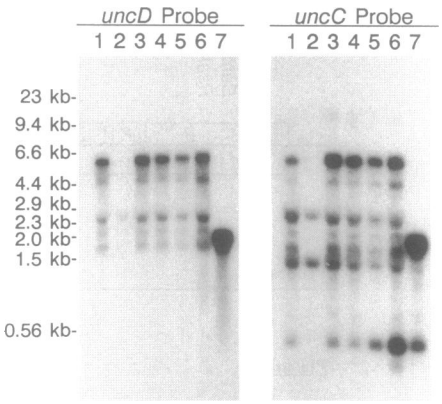

FIG. 3. Northern blot analysis of chromosomally encoded mRNA from various strains. RNA from various strains was subjected to Northern blot analysis with the ^{32}P-labeled *uncD* and *uncC* probes. Lanes: 1, 1100 (*unc*$^+$); 2, DK8 (*unc*); 3, SK5006 (*pnp*$^+$ *rnb*$^+$); 4, SK5005 (*pnp*$^+$ *rnb*); 5, SK5004 (*pnp rnb*$^+$); 6, SK5003 (*pnp rnb*); 7, JM103/pSD13, induced sample. Lanes 1 to 6 received 15 μg of total RNA, and lane 7 received 1 μg of total RNA.

recognized by both probes. Several less-abundant intermediate species were also recognized by both probes, whereas only the *uncC* probe recognized a 0.5-kb species. These fragments of *unc* transcript apparently constitute a nested set with a common 3′ end, presumably the normal 3′ end of the complete transcript, because they are all recognized by the *uncC* probe, which is complementary to the 3′ end of the operon. The 0.5-kb species comigrated with the processed mRNA from plasmid pSD13 (lane 7), implying that the processing event described above for the plasmid-encoded mRNA also occurs in the chromosomally encoded *unc* mRNA. RNA samples prepared from a set of 3′-exonuclease-deficient mutants were also analyzed (lanes 3 to 6). Compared with the wild-type control strain (lane 3), mutations in either RNase II (lane 4) or polynucleotide phosphorylase (lane 5) had little effect on the level of the 0.5-kb *uncC*-specific band, but the double mutant (lane 6) had strongly elevated levels of this species. This result implies that ultimate degradation of the processed fragment is mediated by exonucleolytic decay.

Determination of the 5′ end of the processed transcript. The 5′ end of the processed mRNA was mapped precisely by extension of a primer complementary to the mRNA sequence about 100 bases into the *uncC* gene. cDNA synthesis was carried out by using Moloney murine leukemia virus reverse transcriptase, and the products were electrophoresed alongside the products of dideoxynucleotide sequencing reactions on a sequencing gel (Fig. 4).

Primer extension products obtained by using RNA from strains carrying various recombinant plasmids are shown to the right of the sequencing ladder. Extension products representing the full-length pSD38 mRNA (F) and the processed mRNA (P) are indicated. Note that the processed mRNA from the strain carrying pSD13 has the same 5′ end as that from the strain carrying pSD38. A shorter product (S) present in both of these samples corresponds to a site previously noted by Schaefer et al. (38). Primer extension by RNA transcribed in vitro with T7 polymerase also produced the S band, implying that it represents a stop site for reverse transcriptase owing to secondary structure. The P band was not seen in this sample, implying that it arose from extension of transcripts processed in vivo. The 5′ end of the processed transcript was mapped to be 11 bases into the coding region of *uncC*. The sequence in Fig. 5 shows that the processing removes most of the *uncC* coding sequence from the initiating codon and the ribosome-binding site and at the same time leaves the upstream *uncD* sequence accessible to exonucleolytic attack.

Dependence of the *uncC* processing on RNase E. The recent work implicating RNase E in the processing and degradation of specific mRNAs in *E. coli* prompted us to examine the effect of temperature-sensitive mutations in this enzyme on the cleavage in the *uncC* mRNA. Two RNase E mutant strains, SK5665 (*ams-1*, recently renamed *rne-1*) (3) and N3431 (*rne-3071*), both of which have reduced RNase E activity at the restrictive temperature, were transformed with plasmid pHN1. This plasmid carried the same insert as pSD13, but the parental vector was pEX1 (35), a derivative of pKK223-3 bearing a *lacI*^{q} gene so that expression can be regulated in any strain. RNA was extracted from induced

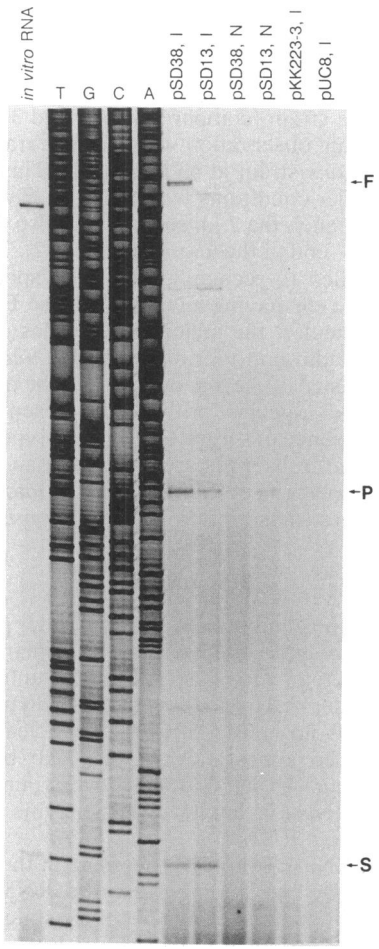

FIG. 4. Primer extension analysis of RNA from strains carrying plasmids pSD38 and pSD13. An end-labeled primer was used to prime cDNA synthesis, using total cellular RNA from *E. coli* JM103 bearing the indicated plasmid as a template. The products were analyzed on a 6% polyacrylamide sequencing gel along with a pSD38-based dideoxy-sequencing ladder. A control (lane labeled *in vitro* RNA) in which the primer was extended on an mRNA transcribed in vitro from pHN6 was used to determine reverse transcriptase stops that were due to secondary structure. The bands corresponding to the full-length (F) transcript of pSD38 and the processed mRNA (P) are indicated on the right. Also shown is a band (S) representing a reverse transcriptase stop. Abbreviations: I, induced samples; N, noninduced samples.

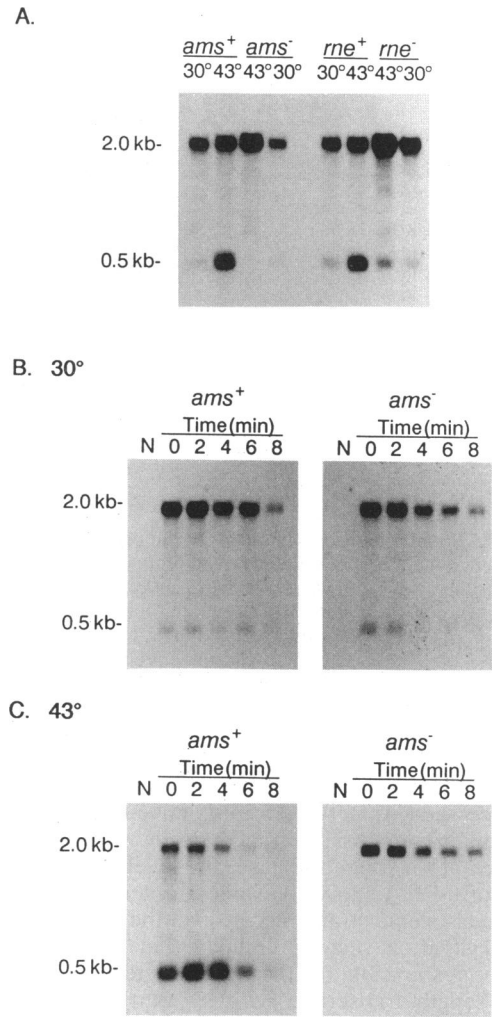

FIG. 6. Analysis of mRNA species transcribed from plasmid pHN1 in various *E. coli* strains. *E. coli* MG1693 (*ams*$^+$), SK5665 (*ams-1*), N3433 (*rne*$^+$), and N3431 (*rne-3071*) bearing pHN1 were grown to early-log phase at 30°C, shifted to 43°C, and induced as described in Materials and Methods. At 10 min after induction, samples were taken for RNA extraction to compare steady-state levels. Rifampin was then added at time zero to block initiation of transcription, and samples were collected for RNA extraction at the indicated time points. Northern blots of the RNA samples were performed by using the DIG-UTP-labeled *uncC* probe as described in Materials and Methods. Abbreviation: N, noninduced samples. (A) Comparison of steady-state mRNA levels. All lanes received 0.44 μg of RNA. (B and C) Degradation of the pHN1 mRNA in MG1693 (*ams*$^+$) and SK5665 (*ams-1*) at 30 and 43°C, respectively.

cells bearing pHN1, and Northern blot analyses were performed with the *uncC*-specific antisense RNA probe (Fig. 6A). Both mutants and their isogenic wild-type strains produced low but detectable levels of the processed fragment at the permissive temperature of 30°C. However, at 43°C each of the mutants contained far lower levels of the 0.5-kb processed mRNA and higher levels of the 2-kb intact mRNA than did the wild-type controls at that temperature. These results imply that RNase E is essential for the processing in the *uncC* mRNA.

FIG. 5. Nucleotide sequence of the intercistronic DNA regions between the *uncD* and *uncC* genes. The *uncD* stop codon, the ribosome-binding site (S-D), and the initiating *uncC* codon are underlined. The position of the processing site is shown by an arrow.

One surprising observation was the extreme elevation of the amount of 0.5-kb product at 43°C compared with 30°C in the RNase E wild-type strains. There were no apparent differences in the levels of the full-length mRNA. Thus, the observed differences imply an increased stability of the processed product at the higher temperature, but the reason for such a striking stabilization is not apparent.

The absence of 0.5-kb cleavage product at the restrictive temperature in the mutants can be most easily explained by

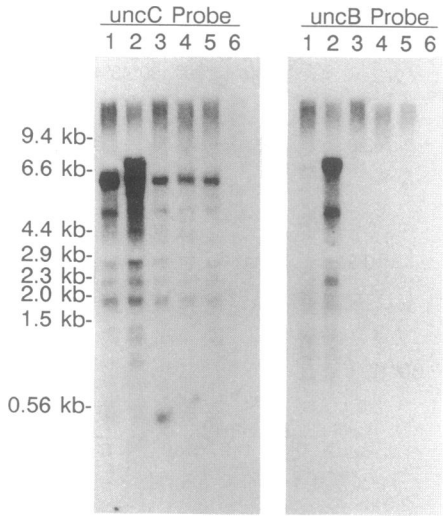

FIG. 7. Requirement of RNase E for the processing of chromosomally encoded *unc* mRNA. Equal amounts of total cellular RNA (10 μg) from various *E. coli* strains were analyzed by Northern blot by using the *uncC*- and the *uncB*-specific antisense RNA probes. Lanes: 1 and 2, SK5665 (*ams-1*) at 30 and 43°C, respectively; 4 and 3, MG1693 (*ams*$^+$) at 30 and 43°C respectively; 5, 1100 (*unc*$^+$); 6, DK8 (*unc*).

an inhibition of the cleavage. An extreme destabilization of the processed product at the restrictive temperature could also explain the disappearance of the smaller products. However, if this were the case, the levels of the full-length precursor mRNA should be same in the mutants and in the wild-type isogenic strain. Because all the strains carried the same plasmid, it seemed unlikely that rates of synthesis would be responsible for the different steady-state levels of the full-length transcript. Analysis of the half-life of the pHN1 mRNA in strains MG1693 (*ams*$^+$) and SK5665 (*ams*) grown at 30°C (Fig. 6B) or 43°C (Fig. 6C) supported a role for RNase E in the processing event. Cells were induced with 1 mM IPTG for 10 min, and then rifampin was added to inhibit further transcription. Northern blot analysis of RNA samples prepared at different time points after addition of rifampin revealed that the 2.0-kb pHN1 mRNA was degraded with a half-life of about 2 min at 30°C in both strains. At 43°C, the half-life was 4 to 5 min in the *ams* strain but just 2 min in the *ams*$^+$ strain. This stabilization in the mutant strain and the lack of significant amounts of the 0.5-kb product imply a role of RNase E in the cleavage. A similar difference in stability was found when the pHN1 transcript was compared in the *rne* and *rne*$^+$ strains (data not shown). The residual degradation of the full-length mRNA in the mutant strains is apparently mediated by the exonucleases, because no stable intermediates can be detected.

Processing of the chromosomally encoded *unc* mRNA in the RNase E mutants. To determine whether the processing in the chromosomally encoded *unc* mRNA is also affected in the RNase E mutant, RNA samples extracted from strains MG1693 (*ams*$^+$) and SK5665 (*ams*) lacking plasmids were analyzed by Northern blots with the *uncC*-specific probe (Fig. 7). The expected 0.5-kb downstream cleavage product was detected at the restrictive temperature in the *ams*$^+$ strain (lane 3) but was not seen in the *ams* strain (lane 2). The mRNA was faintly visible in both strains at the permissive temperature (lanes 1 and 4). This implies that cleavage of the chromosomally encoded *unc* mRNA in the *uncC* coding region is also mediated by RNase E. As in the case of the plasmid-encoded mRNA described above, the level of 0.5-kb fragment is substantially elevated at the higher temperature in the wild-type strain (compare lanes 2 and 3).

One interesting observation was the appearance of a 7-kb species in the *ams* strain at 43°C (lane 2). This species was not present under conditions where RNase E was active. In the right-hand panel, the 7-kb species was also detected with a probe to the 5′ end of the *uncB* gene (lane 2). However, the *uncB* probe failed to recognize any such species in RNA extracted from cells having an active RNase E (lanes 1 and 3 to 5). In particular, the major 6-kb species detected with the *uncC* probe did not hybridize with the *uncB* probe. On the basis of the size of the *unc* operon and the data described above, the 7-kb species probably represents the intact mRNA. The absence of this mRNA in wild-type cells implies that nascent *unc* transcripts are cleaved in an RNase E-dependent reaction at one or more sites in the *uncB* region near the 5′ end before transcription of the operon is complete.

DISCUSSION

The results presented here demonstrate posttranscriptional endonucleolytic processing of the primary *unc* mRNA to produce discrete mRNA species. It is unlikely that the products were derived by 5′-to-3′ exonucleolytic processing, because there is no known 5′-to-3′ exonuclease in *E. coli*. Furthermore, the appearance of the 7-kb band and the absence of the 0.5-kb *uncC* fragment in strains lacking the endonuclease RNase E implicate this enzyme in processing both ends of the mRNA.

The role of RNase E in the processing in the *uncC* region was indicated by the findings that the RNase E mutant (*ams-1* or *rne-1*) contained higher steady-state levels of the pHN1-derived 2-kb mRNA, that this mRNA had a longer half-life, and that the 0.5-kb product was virtually absent. The nucleotide sequence around the *uncC* cleavage site, AAUGA ↓ CUUAC, is also consistent with RNase E involvement (34). Demonstration of a direct role of RNase E in the cleavage will require in vitro studies with a purified preparation of the enzyme.

The residual mechanism of degradation of the 2-kb mRNA in the *ams* mutant produced no stable intermediates, suggesting the involvement of the 3′ exonucleases. We have previously shown that the *unc* terminator hairpin imparts substantial protection against exonucleolytic attack (36); the present results suggest that this protection is not absolute.

The processing of the *uncC* mRNA, about 10 bases into the coding region, has several implications. First, it leads to functional inactivation of the *uncC* mRNA by removing the ribosome-binding site and the initiating codon from the rest of the coding region. The cleavage is interesting because it occurs at a site that will minimize the stranding of ribosomes on a faulty mRNA. It creates a truncated mRNA species which cannot load ribosomes, and translationally inactive mRNAs may be more prone to degradation (5). Second, the absence of any upstream product of the cleavage must reflect a very short half-life. It seems most likely that exonucleolytic attack at the new 3′ end, which lacks a terminator hairpin, rapidly degrades the *uncD* region.

In accordance with the subunit stoichiometry of F_1F_0-ATPase, *uncD* is more highly expressed than *uncC*. However, the processing has no apparent role in controlling the differential expression of the β and ε subunits because it leads to inactivation of both *uncD* and *uncC* genes. More

probably, this processing may be an important event in the overall degradation of the *unc* mRNA. In support of this idea, the total levels of *unc* mRNA were markedly increased in the *ams* mutant. The relative importance of the *uncC* site to any others is not certain, but this site is well situated to initiate the exonucleolytic degradation of the upstream *unc* mRNA, as well as inactivating the final *uncC* cistron directly.

Using RNA extracted from the RNase E mutant, we have detected a 7-kb chromosomally encoded species which we believe to be the full-length *unc* transcript. This mRNA is of the expected size and hybridizes to probes to both the 5′ (*uncB*) and the 3′ (*uncC*) regions of the operon. The 7-kb species is hardly detectable in *ams*$^+$ strains, implying that it is rapidly processed in wild-type cells to produce the 6.2-kb species that hybridizes with *uncC* but not with *uncB*. On the basis of the in vivo mRNA elongation rate of 42 nucleotides per s (15), transcription of the *unc* operon should require 2 to 3 min. This allows ample time for processing a site near the 5′ end before transcription of the 3′ end is completed.

Previously, chromosomally encoded *unc* mRNA has been studied by Jones et al. (18), by McCarthy et al. (27), and by Schaefer et al. (38). Our results agree with those of the last two groups in demonstrating the presence in wild-type cells of a major 6.2-kb mRNA lacking the 5′ end of the operon. The studies with the *ams* mutant extend their findings by demonstrating the existence of the precursor 7-kb mRNA that includes *uncB* and implicating RNase E in its conversion to the 6.2-kb species. Recently, full-length plasmid-borne mRNA has been detected by McCarthy et al. (25).

The processing in the 5′ end of the *unc* mRNA may be important in the differential expression of some *unc* genes. The upstream *uncIB* mRNA is apparently degraded, because no species of the appropriate size was detected with the *uncB* probe. These results correlate well with the recent demonstration by McCarthy et al. (25) that the functional half-lives of the *uncI* and *uncB* mRNAs are just 1 to 2 min, compared with 5 to 12 min for the third gene in the operon, *uncE*. The almost complete absence of the 7-kb transcript in *rne*$^+$ cells (Fig. 7) implies that the physical half-lives of *uncI* and *uncB* are short relative to the 2 to 3 min required to synthesize the complete mRNA. The rapid degradation of the 5′ end can be expected to restrict the levels of proteins expressed from the first two genes. It should be noted in this regard that the *uncB* gene product, the hydrophobic *a* subunit, occurs in just one copy per ATP synthase molecule and is known to be toxic if overexpressed (12).

Two RNase E cleavage sites have recently been identified by Gross (16) in the *uncBE* intercistronic region of hybrid plasmid-encoded mRNAs. We have identified the same sites in chromosomally encoded *unc* mRNA extracted from *rne*$^+$ cells (37). The 5′ ends produced by cleavage at these sites are located 27 and 45 bases upstream from the *uncE* initiation codon.

Some of the other species seen on Northern blots, such as the 5-kb species detected with the *uncC* probe, were not affected by the *ams* mutation. If these arise through endonucleolytic processing, it seems likely that other nucleases are involved.

In conclusion, our studies demonstrate that both ends of the 7-kb *unc* mRNA are subject to RNase E-dependent endonucleolytic processing. The 5′ processing events appear to be involved in reducing the half-lives of the first two genes, whereas we speculate that the 3′ event may initiate degradation of the major 6-kb mRNA that remains. The processing sites in the 5′ end and the functional implications of both 5′ and 3′ processing events are under continuing investigation.

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