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mRNA processing independent of RNase III and RNase E in the expression of the F1845 fimbrial adhesin of *Escherichia coli*

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ABSTRACT **F1845, the fimbrial adhesin of a diarrhea-associated *Escherichia coli*, confers upon the bacteria the ability to adhere to cultured epithelial cells in a diffuse pattern. The fimbrial subunit gene, *daaE*, is encoded on a polycistronic mRNA which is processed endoribonucleolytically to produce a stable message encoding only *daaE*. The processing event occurs in bacterial strains with mutations in RNase III or RNase E, the only endoribonucleases which have been implicated in the processing of *E. coli* mRNA. Sequences encoding a stem–loop structure downstream of *daaE* play an essential role in determining the stability of the *daaE* mRNA. Rapid degradation of the sequences upstream of the cleavage site occurs upon processing, suggesting that processing of the F1845 polycistronic mRNA results in differential expression of genes involved in the biogenesis of fimbriae.**

The F1845 fimbrial adhesin confers upon *Escherichia coli* the ability to adhere to cultured epithelial cells in a diffuse pattern. The determinant encoding expression of F1845 was isolated from the chromosome of an *E. coli* strain obtained from an infant with persistent diarrhea and consists of at least six genes, designated *daaA–daaF* (1, 2). The gene encoding the major fimbrial subunit, *daaE*, is located at the 3′ end of the locus. The major fimbrial subunit also serves as the adhesin (3). F1845 is a member of a family of related adhesins including AFA/I and the Dr hemagglutinin, which recognize the Dr(a) blood group antigen as a receptor on human erythrocytes (1, 3, 4).

Assembly of *E. coli* fimbriae depends on the regulation of appropriate levels of major and minor fimbrial subunits, assembly proteins, and regulatory proteins relative to one another. The major fimbrial subunit, several hundred of which constitute a single fimbria, must be expressed at higher levels than minor subunits and other accessory proteins (5, 6). We are investigating the transcriptional organization of the F1845 determinant in an effort to understand regulatory mechanisms involved in the differential expression of genes which constitute the determinant.

In this report, the 5′ terminus of a 1.3-kb mRNA transcript encoding *daaE*, the F1845 major subunit gene, is defined.† This transcript is shown to be a highly stable product of processing of a larger polycistronic mRNA encoding additional genes of the F1845 determinant. The endoribonucleolytic cleavage which generates the *daaE* transcript, in contrast to other *E. coli* mRNA processing events described to date, requires neither RNase III nor RNase E.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Media. Strain C1845 is a clinical *E. coli* isolate from a child with protracted diarrhea (1). *E. coli* DH5α (Bethesda Research Laboratories) was used as a host strain for plasmid constructs, and strain GW5180 (a *recA* derivative of strain JM101) was used as a host for bacteriophage M13 derivatives in nucleotide sequence analysis and for oligonucleotide-directed mutagenesis experiments (7). *E. coli* strain RZ1032 (*dut ung*) was used as a host to propagate M13 phage for oligonucleotide-directed mutagenesis (8). *E. coli* strains W3110 and NC124 (W3110 *rnc-105 glyA*::Tn*5*) (9), kindly provided by Donald L. Court, were used to examine the role of RNase III in *daaE* mRNA processing. *E. coli* strains MG1693 and SK7669 (MG1693 *rne-3071*) (10), kindly provided by Sidney R. Kushner, were used to examine the role of RNase E in *daaE* mRNA processing. pUC18 and pUC19 (11) were used as cloning vectors. A promoterless *lacZ* gene was isolated from plasmid pSK202 (12) and used for construction of *lacZ* transcriptional fusions. Plasmid constructs of the *daa* operon were derived from pSSS1, which encodes the entire determinant (1). Plasmid pMAA100 was constructed by inserting a 2.0-kb *Sst* I–*Xho* I fragment of pSSS1 containing *daaE* into pUC18 so that the *lac* promoter (P_{lac}) was located upstream of the *Sst* I site. Construction of other plasmids is described in the text. Bacteria were grown in Luria broth or on Luria agar plates (13) at 37°C unless otherwise indicated. Ampicillin and spectinomycin were used at a final concentration of 100 μg/ml.

DNA and RNA Methods. Methods used in DNA isolation, recombinant DNA methods, sequence analysis, RNA isolation, Northern blot analysis, and primer extension analysis are described in detail elsewhere (2).

Oligonucleotide-Directed Mutagenesis. Oligonucleotide-directed site-specific *in vitro* mutagenesis was performed by the procedure of Kunkel (14). Synthetic oligonucleotide primers were prepared on a Biosearch 8600 DNA synthesizer (Biosearch).

***In Vitro* RNA Processing with RNase III.** Thirty micrograms of RNA was treated with 40 ng of purified RNase III (kindly provided by Donald L. Court) in the presence of 50 mM Tris·HCl (pH 8.0), 150 mM NaCl, and 10 mM $MgCl_2$ at 37°C for 30 min. The products of the reaction were ethanol-precipitated and analyzed by primer extension.

S1 Nuclease Protection Experiments. Probes were prepared and nuclease protection experiments were performed as described elsewhere (2). For the experiments reported here, an *Sst* I–*Xho* I fragment of pSSS1 containing *daaE* was inserted into the *Sst* I–*Sal* I sites of M13mp18. This construct was designated SX2. An *Eco*RI site was introduced by site-directed mutagenesis at a position upstream of the 5′ terminus of the *daaE* transcript (see text), and this construct was designated SX2E. An *Eco*RI fragment bounded by the *Eco*RI site in the M13mp18 polylinker and the introduced *Eco*RI site upstream of *daaE* was deleted, resulting in SX2EΔE. Templates for S1 probes were derived from this construct.

Determination of mRNA Half-Life. Cultures of *E. coli* were grown to midlogarithmic phase, at which point rifampicin (0.5

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M27725).

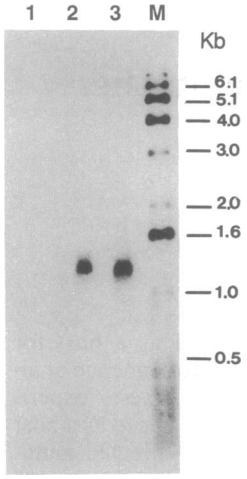

FIG. 1. Analysis of *daaE* transcription by Northern blot hybridization. RNA samples from *E. coli* strains DH5α(pUC18) (10 μg) (lane 1), C1845 (20 μg) (lane 2), and DH5α(pSSS1Ω) (10 μg) (lane 3) were hybridized to a ^{32}P-labeled *Pst* I DNA fragment derived from the region encoding the 5′ end of *daaE*. ^{32}P-end-labeled DNA fragments were used as molecular size markers (lane M).

mg/ml) was added to block further initiation of transcription. Culture samples were then withdrawn at various time points, total RNA was extracted, and the level of specific mRNA was determined by quantitative primer extension analysis. Dot blot analysis of the RNA samples was performed (15) with a DNA probe labeled with [α-^{32}P]dCTP (NEN) by the random primer method (16). The relative decay rate of the *daaE* transcript was determined by densitometric scanning of the autoradiograms (model 60 Visage system scanner; BioImage, Ann Arbor, MI).

Determination of β-Galactosidase Activity. The specific activity of β-galactosidase was determined as described by Miller (13). Values were obtained from at least three independent experiments in which β-galactosidase activity was assayed in triplicate.

RESULTS

Analysis of *daaE* Transcription by Northern Blot Hybridization. The entire F1845 determinant is encoded by pSSS1. *daaE* is the terminal gene of the determinant and encodes the major fimbrial subunit (1). To determine the size and number of transcripts expressing *daaE*, the omega fragment (17) was inserted in pSSS1 at a *Bam*HI site upstream of all known genes of the determinant. This fragment contains an efficient transcriptional terminator; thus any vector-originated transcription would be blocked. This plasmid was designated pSSS1Ω. RNA extracted from strains C1845, DH5α(pSSS1Ω), and DH5α(pUC18) was analyzed by Northern blot hybridization using a 375-base *Pst* I fragment encoding the 5′ end of *daaE* as probe (Fig. 1). Both wild-type strain C1845 and DH5α(pSSS1Ω) expressed the *daaE* gene on a transcript of ≈1.3 kb (Fig. 1, lanes 2 and 3). The probe did not hybridize to RNA from DH5α(pUC18) (lane 1). Longer exposure of the Northern blots revealed binding of the probe to larger RNA species which were not resolved in a discrete band (data not shown). A probe from *daaC*, which is upstream of *daaE*, similarly hybridized to larger RNA species which were not resolved in discrete bands (data not shown).

Mapping the 5′ Terminus of the *daaE* Transcript. To define the promoter and regulatory elements involved in the expression of *daaE*, the 5′ terminus of the *daaE* transcript was determined by primer extension and S1 nuclease protection analyses of RNA extracted from strains C1845 and DH5α(pSSS1). Primer extension analysis using primer 690 (Fig. 2) detected two 5′ termini corresponding to positions 113 and 236 upstream of the *daaE* initiator codon (Fig. 3). Primer 722 (Fig. 2) was used in an additional primer extension to precisely define the terminus of the longer extension product. S1 nuclease protection experiments identified only a single terminus, 237 bases upstream of the *daaE* initiator codon (data not shown), indicating that the shorter fragment detected by primer extension analysis resulted from premature termination of the extension reaction. The results of these experiments located the 5′ terminus of the *daaE* transcript at 236 or 237 bases upstream of the *daaE* initiator codon (Fig. 2).

***daaE* mRNA Is the Processing Product of a Larger Transcript.** Sequences resembling consensus promoter −10 and −35 sequences were not found in the DNA region upstream of the 5′ terminus of the *daaE* mRNA. This observation suggested the possibility that the *daaE* transcript may not originate at the identified 5′ terminus but, rather, result from an endoribonucleolytic cleavage at that site. This hypothesis was tested by analyzing the effect of omega insertions on the level of *daaE* transcription by using a *daaE–lacZ* transcriptional fusion (Fig. 4). pSSS9Ωz, which carries an omega insertion in the *Bam*HI site to block transcription initiated from P_{lac}, and pSSS9.2Ωz, which carries an omega insertion in the *Eco*RV-1 site, expressed approximately equivalent levels of β-galactosidase activity. However, the level of β-galactosidase activity expressed from pSSS9.3Ωz, carrying an omega insertion in the *Eco*RV-2 site, was only one-ninth that expressed from pSSS9Ωz. These experiments indicated that transcription of *daaE* mRNA is dependent on a promoter in the 700-bp *Eco*RV fragment upstream of *daaA*. The efficiency of the transcriptional terminator of the omega fragment is demonstrated by analysis of pSSS9.6Ωz and pSSS9.7Ωz, which place the *daaE–lacZ* gene fusion under the control of P_{lac} in close proximity. Insertion of the omega fragment upstream of the fusion reduces β-galactosidase activity by a factor of almost 1000. The low but detectable level of β-galactosidase activity detected from pSSS9.3Ωz as

FIG. 2. Nucleotide sequence of the DNA regions upstream and downstream of the *daaE* open reading frame. The sequence of one strand in the 5′-to-3′ direction starting 309 bases upstream of the translational start codon and ending 94 bases downstream of the translational stop codon of *daaE* is shown. Primers 690 and 722 were complementary to the sequences indicated by the bars. Vertical arrows indicate the site encoding the 5′ terminus of the *daaE* transcript as determined by S1 nuclease protection analysis (s) or by primer extension analysis (p). The long horizontal arrow indicates the 5′ end and the direction of transcription of the *daaE* mRNA. Restriction sites shown in parentheses were introduced at the indicated sites after altering bases by oligonucleotide-directed mutagenesis (see text). The paired arrows show sequences that have the potential to encode a stem–loop structure.

compared with pSSS9.7Ωz (Fig. 4) is attributed to the 5-kb region between the omega fragment and the *daaE–lacZ* fusion in pSSS9.3Ωz, where spurious transcriptional initiation may occur. These results indicated that the *daaE* mRNA is the processing product of a larger precursor message whose transcription is initiated upstream of the *Eco*RV-2 site (Fig. 4). However, Northern blot analysis did not detect a discrete large unprocessed transcript, suggesting that the processing event is very rapid and/or that the precursor mRNA is unstable.

Detection of the Unprocessed Precursor Transcript. Additional S1 nuclease protection experiments were performed with a probe containing M13 sequence at its 3′ end in order to enable differentiation between undigested excess probe and probe protected by an unprocessed precursor transcript. These experiments resulted in the detection of a number of fragments (Fig. 5). A 500-base fragment corresponding to the full-length undigested probe [seen at the top of Fig. 5 *C* and *D* (this region is not shown in *B*)] and a 114-base fragment corresponding to the 5′ end of the processed *daaE* mRNA were detected as expected. A 185-base protected fragment corresponding to the entire *daa*-derived portion of the probe indicated the presence of an unprocessed precursor transcript encoding *daaE*. For unknown reasons, this fragment was present at higher levels when RNA from the clinical isolate C1845 was analyzed (Fig. 5*B*, band P) compared with RNA from strains carrying recombinant constructs (Fig. 5 *C* and *D*). This suggests that processing may be less efficient in the wild-type strain. A 235-base protected fragment was detected when the probe was hybridized to RNA extracted from strains carrying pSSS1Ω (Fig. 5 *C* and *D*, band V). This fragment was determined to have resulted from the protection of the M13-derived 3′ region of the probe by vector-encoded transcripts, since this fragment was also seen as the sole protected fragment in control assays using RNA from strains carrying pUC derivatives with no insert (data not shown). In these S1 nuclease protection experiments, the failure to detect a protected 71-base fragment corresponding to the 3′ terminus of the processed transcript indicates that after processing, the upstream cleavage product is much less stable than the downstream product encoding *daaE*.

Processing of *daaE* mRNA in an RNase III-Deficient Strain. To investigate the possible role of RNase III in the processing of the *daaE* mRNA, the production of this message by pSSS1Ω in the RNase III mutant strain NC124 (*rnc-105*) and its parent strain W3110 was analyzed. Total RNA was extracted from logarithmic-phase cultures, and production of *daaE* mRNA was analyzed by primer extension (data not shown) and S1 nuclease protection (Fig. 5). The processed *daaE* mRNA was detected in the analyses of RNA extracted from the RNase III mutant as well as the wild-type strain, and no accumulation of precursor was detected in the RNase III mutant. Primer extension experiments also demonstrated correct processing by the RNase III mutant (data not shown). As a control, the same RNA samples were also used in primer extension analysis for detecting the 5′ end of the transcript encoding the polynucleotide phosphorylase gene (*pnp*), which has been shown to be processed by RNase III (18). These experiments confirmed that the *pnp* message was not processed at the expected site in the RNase III mutant. Correct processing of this message was accomplished by the addition of purified RNase III to RNA obtained from the RNase III mutant (data not shown). These results indicate

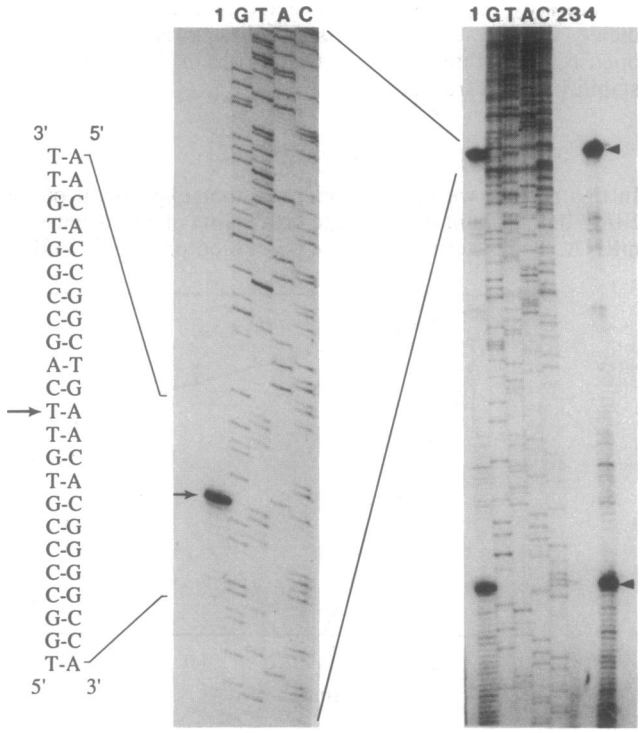

FIG. 3. Primer extension analysis of the *daaE* transcript. Primer 722 (*Left*) or 690 (*Right*) was annealed to RNA isolated from *E. coli* DH5α(pSSS1) (lanes 1 and 4), C1845 (lane 2), and DH5α (lane 3) and extended with murine leukemia virus reverse transcriptase. See Fig. 2 for location of primers. A dideoxy sequencing ladder generated by using the same primers was run as size standards (lanes G, T, A, and C). Arrows indicate identified termini.

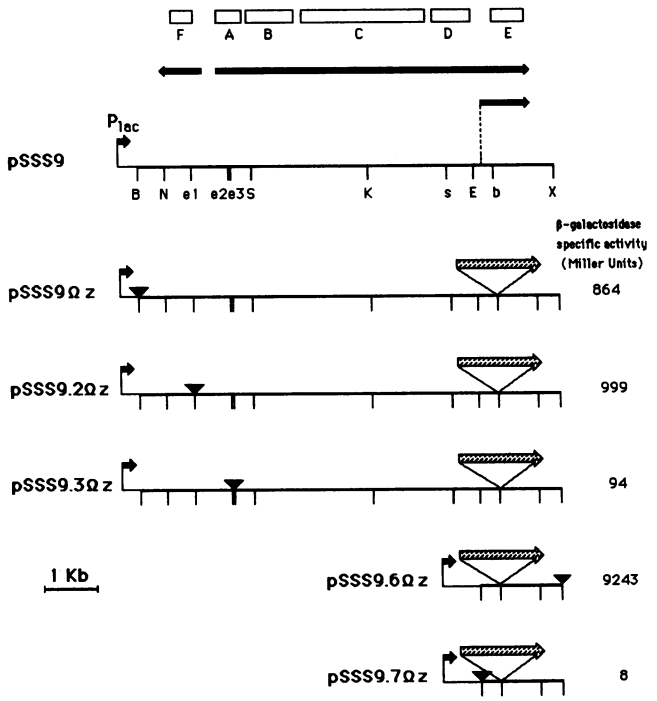

FIG. 4. Expression of β-galactosidase by *daaE–lacZ* transcriptional fusions. The top line shows the genetic and physical map of the F1845 determinant on pSSS9 which was used to construct the *daaE–lacZ* fusions. Arrows above pSSS9 indicate the 5′ end and direction of transcripts encoded by the F1845 determinant (1, 2). Peptides encoded by the F1845 determinant are indicated by boxes above the regions where they are encoded. Solid triangles indicate the location of omega-fragment insertions. Hatched arrow indicates the location and orientation of the promoterless *lacZ* gene. Expression levels of β-galactosidase activity in *E. coli* DH5α harboring the various fusions are shown at right. Restriction enzyme sites: B, *Bam*HI; b, *Bgl* II; e, *Eco*RV; E, *Eco*RI; K, *Kpn* I; N, *Nsi* I; S, *Sph* I; s, *Sst* I; X, *Xho* I.

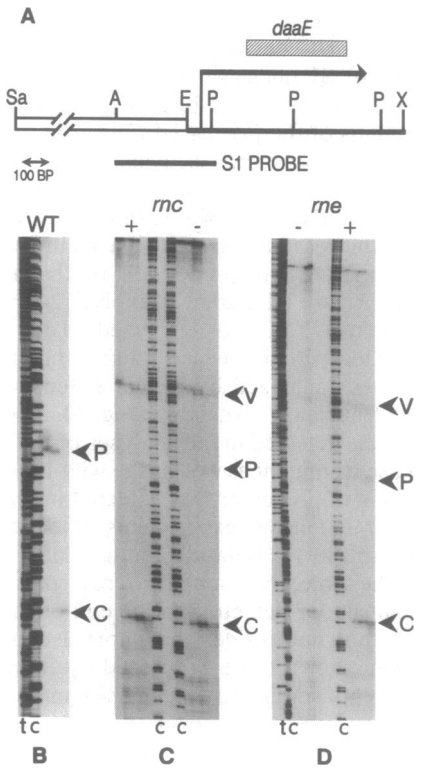

FIG. 5. S1 nuclease protection analysis of the *daaE* transcript. (*A*) Probe used for S1 nuclease protection analysis. The M13 clone SX2EΔE (see *Materials and Methods*) used to construct single-stranded DNA probes is shown: open bar represents M13 sequence, *daa* sequence is shown by a solid line. Long horizontal arrow indicates the 5′ end and the direction of transcription of the *daaE* mRNA and the hatched bar above it represents the *daaE* coding region. The probe included 301 bases of M13 in addition to F1845-related sequences. Restriction enzyme sites: A, *Ava* II; P, *Pst* I; Sa, *Sal* I; X, *Xho* I. Restriction site for *Eco*RI (E) was introduced by oligonucleotide-directed mutagenesis. (*B*) Probe was hybridized to RNA extracted from *E. coli* strain C1845 and digested with 10 units, 30 units, and 90 units of S1 nuclease, loaded left to right. (*C*) Probe was hybridized with W3110(pSSS1Ω) RNA (*rnc*$^+$) and NC124(pSSS1Ω) RNA (*rnc*$^-$). The reaction mixtures were digested with 3, 10, 30, and 90 units of S1 nuclease, loaded left to right. (*D*) Cultures of strains MG1693 (*rne*$^+$) and SK7669 (*rne-3071*) harboring pSSS1Ω were grown to logarithmic phase at 30°C, then shifted to 44°C for 70 min before RNA was extracted, annealed to the probe, and digested with 3, 10, 30, and 90 units of S1 nuclease, loaded left to right. The T and C dideoxy sequencing ladders of M13 clone SX2EΔE with the same primer used to generate the S1 probe were electrophoresed with the S1 reaction mixtures in order to determine the length of the protected fragments (lanes t and c). Fragments C represent probe protected by processed *daaE* transcripts, fragments P are protected by precursor *daaE* transcripts, and fragments V are protected by vector-encoded transcripts. Reactions from mutant and wild-type strains in *C* and *D* were loaded at slightly different times to limit diffusion; thus bands V, P, and C are offset in these panels.

that RNase III is not involved in the processing of the *daaE* mRNA.

Processing of *daaE* mRNA in an RNase E-Deficient Strain. To test the possible role of RNase E in the processing of the *daaE* mRNA, cultures of the conditional RNase E mutant strain SK7669 (*rne-3071*) and its parent strain MG1693 carrying plasmid pSSS1Ω were grown to logarithmic phase at 30°C, then shifted to 44°C for 70 min. The *rne* mutation was confirmed by the inability of SK7669 to grow at 44°C. RNA was then extracted and analyzed by S1 nuclease protection (Fig. 5). No accumulation of unprocessed transcript was observed in the RNase E mutant strain relative to the wild-type strain. Furthermore, Northern blot analyses of the RNA extracted from the RNase E mutant strain and its wild-type parent strain detected equivalent levels of the processed *daaE* transcript and no accumulation of higher molecular weight precursor RNA species (data not shown). These results therefore indicate that RNase E does not play a role in the cleavage reaction which generates the *daaE* transcript.

Stability of the *daaE* Transcript. To measure the half-life of the processed *daaE* mRNA, RNA was isolated from a logarithmic-phase culture of DH5α(pMAA100) at various times after transcription had been blocked with rifampicin. This plasmid places *daaE* under the control of P_{lac}. The RNA was analyzed by dot blot hybridization with a 450-bp *Pst* I fragment encoding the 3′ end of *daaE* (1). The half-life of the *daaE* mRNA was estimated by densitometric analysis of autoradiograms to be ≈22.5 min (Fig. 6).

Upon examination of sequences distal to the *daaE* open reading frame, a region of dyad symmetry that would allow the formation of a stem–loop structure at the 3′ end of the *daaE* mRNA was identified 55 bp downstream of the stop codon (Fig. 2). To investigate the possible role of this region in the stability of the *daaE* transcript, the bases encoding the 3′ stem–loop were altered by oligonucleotide-directed mutagenesis, yielding pMAA101. Primer 1482 (5′-GGCAGCG-CACCTCTAGATAGACCTCTTGTGTTC-3′) changed nucleotides GGGGC starting 55 bases downstream of the *daaE* stop codon to TCTAG, thus introducing a unique *Xba* I site. The effect of these mutations on the half-life of *daaE* mRNA was determined by dot blot analysis. The half-life of the mutant *daaE* mRNA was estimated by densitometric analysis of the autoradiograms to be 4.7 min compared with 22.5 min for the wild-type *daaE* mRNA (Fig. 6). Similar values were obtained by quantitative primer extension analysis of these RNA samples. There was thus a factor-of-4 reduction in the half-life of the *daaE* mRNA after disruption of the stem–loop structure by oligonucleotide-directed mutagenesis. This indicates that the stem–loop structure downstream of the *daaE* open reading frame plays a crucial role in determining the stability of this transcript.

DISCUSSION

In this report, we present evidence that expression of the F1845 determinant involves the processing of a polycistronic mRNA. The 5′ terminus of the processed product encoding

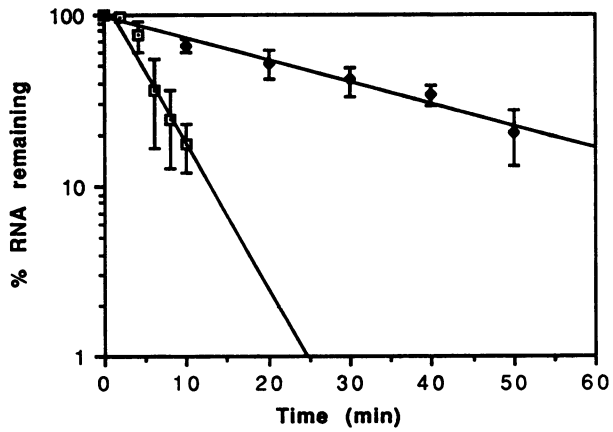

FIG. 6. Kinetics of the wild-type and mutant *daaE* mRNA decay in rifampicin-treated cells. The relative amounts of *daaE* mRNA, extracted from DH5α carrying pMAA100 (●) or pMAA101 (□) at various times after addition of rifampicin were determined by densitometric scanning of autoradiograms from RNA dot blots probed with a ^{32}P-labeled *daaE*-specific DNA probe. The amount of RNA in the zero-time sample was set to 100%. The values plotted represent the mean ± SD from three separate experiments.

daaE mRNA was defined. Insertional mutagenesis of F1845 sequences using the omega fragment indicated that the expression of a *daaE–lacZ* transcriptional fusion was dependent on a promoter located several kilobases upstream. A promoter has been identified at this site (2). These data indicate that the 5′ terminus of the *daaE* transcript results from the site-specific endoribonucleolytic cleavage of a precursor polycistronic mRNA. Since no 5′ → 3′ exoribonucleolytic activity has been described in *E. coli*, we presume that the 5′ terminus of the *daaE* transcript is the cleavage site. The failure to detect the 3′ end of RNA products corresponding to regions upstream of the cleavage site by S1 nuclease protection analysis suggests that following processing, the upstream products are rapidly degraded. Our inability to detect discrete transcripts of genes upstream of *daaE* by Northern blot analysis is consistent with this hypothesis. In contrast, the processed *daaE* mRNA was determined to have an exceptionally long half-life. Sequences downstream of the *daaE* stop codon encode a potential stem–loop structure in the *daaE* transcript which may contribute to this stability. We propose that segmental differences in the stability of the *daa* transcripts generated by endoribonucleolytic processing result in the posttranscriptional regulation of genes involved in F1845 expression.

Extensive sequence homology between regions upstream of *daaE* and regions upstream of the structural subunit genes of two uropathogenic *E. coli* hemagglutinins, Dr and AFA/I (1, 3, 19), suggests that RNA processing may also be involved in the expression of these adhesins. Primer extension analyses of the transcripts encoding *draA* and *afaE* demonstrated that the 5′ terminus of these transcripts mapped to a sequence identical with the sequence at the *daaE* cleavage site (unpublished observations). Stabilizing stem–loop structures downstream of the major fimbrial subunit-encoding sequences may also be a common feature of this family of adhesins. While nucleotide homology between *daaE* and *draA* diverges in the region encoding the mature protein, the sequence 54 bp downstream of the *draA* stop codon (unpublished data) revealed nucleotide homology to the region downstream of the *daaE* stop codon which was defined to be essential to the stability of the *daaE* mRNA.

Endoribonucleolytic cleavage is thought to be the initial step in the decay pathway of many transcripts (20). Since this type of regulation has previously been shown in the *papBA* operon of the P fimbrial determinant (21), RNA processing and differential stability may be a common mechanism of posttranscriptional regulation of gene expression among fimbrial adhesin determinants of *E. coli*. Processing of *pap*-encoded transcripts is the rate-limiting step in the decay of *papB* mRNA, which encodes a DNA-binding regulatory protein (22). In contrast to the *daaE* transcript processing event, the cleavage of the *papBA* transcript results from an RNase E-dependent site-specific cleavage at sequences that show homology to the RNase E cleavage motif (G-A/U-cut-A-U-U) (23). S1 nuclease protection experiments suggest that the processing of *daaE* mRNA is the rate-limiting step in the decay of mRNA upstream of the cleavage site. Sequences flanking the *daaE* mRNA cleavage site (C-U-cut-G-A-A, or U-G-cut-A-A-C), as detected by S1 nuclease protection or by primer extension analysis, respectively, share little homology with the RNase E cleavage motif. Consistent with this observation, our results show that RNase E has no role in the processing of the *daaE* mRNA. This indicates that different endoribonucleases are involved in the processing of transcripts expressed by different fimbrial adhesin determinants. Furthermore, it was also demonstrated that RNase III was not involved in the processing of the *daaE* transcript. Since RNase III and RNase E are the only endoribonucleases of *E. coli* that have been shown to have a role in the processing of specific mRNA substrates (9, 10, 18, 21, 24–27), our results raise the intriguing possibility that a novel endoribonuclease may play an important role in the regulation of gene expression in *E. coli*.

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