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Differential Decay of RNA of the CFA/I Fimbrial Operon and Control of Relative Gene Expression

BART J. A. M. JORDI,* INGRID E. L. OP DEN CAMP, LOUISE A. M. DE HAAN, BERNARD A. M. VAN DER ZEIJST, AND WIM GAASTRA

Institute of Infectious Diseases and Immunology, Department of Bacteriology, Faculty of Veterinary Medicine, University of Utrecht, 3508 TD Utrecht, The Netherlands

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CFA/I fimbriae on human enterotoxigenic *Escherichia coli* are composed of the CfaB protein, the product of the second gene of the CFA/I operon. We show here that CfaB is expressed at a higher level than other proteins of the CFA/I operon. mRNA encoding the CfaB protein is much more abundant than mRNA encoding CfaA, the first protein, together with CfaB or mRNA encoding CfaA only. Only one promoter, upstream of *cfaA*, is present. These data indicate that a primary transcript containing *cfaA* and *cfaB* is processed into a *cfaA*-specific mRNA and a *cfaB*-specific mRNA. The *cfaA* mRNA is unstable, while the *cfaB* mRNA is stable and therefore accumulates in CFA/I-producing *E. coli*. The *cfaB* mRNA is probably stabilized by a stem-loop structure downstream of the *cfaB* gene. No distinct mRNA fragments could be detected encoding the other two proteins, CfaC and CfaE, of the CFA/I operon. These results indicate that *cfaC*-and *cfaE*-specific mRNAs degrade very rapidly and/or are produced in small amounts.

Enterotoxigenic *Escherichia coli* (ETEC) strains are an important cause of travellers' diarrhea and of endemic diarrhea in infants in developing countries (23). ETEC strains colonize the small intestine with the aid of adhesion factors, after which they cause diarrhea by producing enterotoxins. The adhesion factors are often fimbriae. A single fimbria is a long, threadlike proteinaceous structure composed of hundreds of identical subunits.

On human ETEC strains, several serologically different variants of fimbriae are known (i.e., CFA/I, CFA/III, CS1, CS2, CS3, CS4, CS5, and CS6; see reference 17). In strain E7473/0, the genes coding for the proteins needed for expression of the CFA/I fimbriae are located on the same plasmid that encodes the enterotoxin ST (9, 28). On this CFA/I-ST plasmid (NTP113), two regions, separated by about 40 kb, are needed for production of CFA/I fimbriae (29). These regions (regions 1 and 2), when cloned separately on compatible vectors, complement each other, resulting in CFA/I production by the *E. coli* K-12 host (33).

Region 1 contains four open reading frames needed for CFA/I production, designated *cfaA*, *cfaB*, *cfaC*, and *cfaE* (14). The *cfaB* gene encodes the subunit protein, whereas the function of the *cfaC* and *cfaE* gene products is unknown. The *cfaA* gene product is very homologous with the CooB protein (also designated CsoB [13]), and since CooB is needed for assembly of CS1 fimbriae (27), the CfaA protein is probably also needed for assembly of fimbriae. Region 2 contains one open reading frame designated *cfaD* (or *cfaR* [4]), which is needed for CFA/I production (25). The *cfaD* gene encodes a protein which is a member of the AraC family of positive regulators. The function of the CfaD protein is to overcome the repression mediated by histone-like protein H-NS at the promoter in front of the *cfaA* gene in CFA/I region 1 (12).

The subunit protein CfaB is much more abundant than other gene products of region 1 in an in vitro transcription-translation assay. Here, we demonstrate that this differential expression of genes from CFA/I region 1 is probably regulated by differential stability of mRNA fragments of this region.

Several processes regulate the stability and degradation of mRNA in bacteria: in *E. coli*, two 3′ to 5′ exoribonucleases, PNPase and RNase II (6, 7), degrade mRNA by removing ribonucleotides one by one from the 3′ end of the transcripts; no 5′ to 3′ exonucleolytic activity is known. Processing of transcripts by site-specific endoribonucleases produces new 3′ ends which are susceptible to degradation by 3′ to 5′ exoribonucleases (3). Stem-loop structures are believed to block 3′ to 5′ exoribonucleases, resulting in the stabilization of upstream mRNA (3).

The role of the processes described above in the differential stability of mRNA fragments of CFA/I region 1 is discussed.

MATERIALS AND METHODS

Bacterial strains and plasmids. Strain BL321 is an RNase III-negative (*rnc-105*) *E. coli* strain, and strain BL322 is its RNase III-positive isogen (31). *E. coli* N3431 contains a temperature-sensitive mutation (*rne-3071*), making it RNase E deficient at 44°C; strain N3433 is its wild-type isogen (1). *E. coli* NHY312 is a derivative of strain NHY322 containing a temperature-sensitive mutation (*rnpA49*) in the gene coding for protein C5 of the holoenzyme RNase P (16). *E. coli* HMG11 (10) was used as a host in the β-galactosidase assays. *E. coli* E7473/0 (28) was used as a CFA/I-producing strain.

Plasmids NTP513 (a pACYC184 derivative containing CFA/I region 1 [33]), pIVB3-105 (a pACYC184 derivative containing *cfaD* [25]), pIVB3-100 (a Bluescript M13$^+$ derivative containing *cfaD* [25]), and pIVB3-710 (a pCB267 derivative containing the promoter of the *cfaA* gene cloned in front of *lacZ* [12]) have been described previously. Plasmid pIVB3-529, a derivative of plasmid pCB267 (26), was constructed by digesting and ligating a polymerase chain reaction (PCR) product of 260 bp (see oligonucleotides B1 and B2 in Fig. 5) in the *Bam*HI and *Sal*I sites of the vector.

Bacteria used for isolation of RNA were grown in CFA medium (8) and harvested at an optical density at 600 nm of

* Corresponding author. Present address: Imperial Cancer Research Fund, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, United Kingdom OX3 9DU.

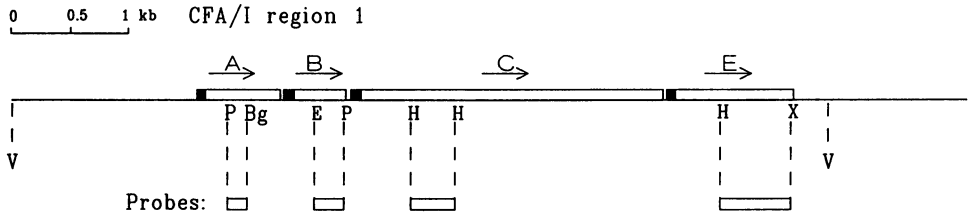

FIG. 1. CFA/I region 1. The *cfaA*-, *cfaB*-, *cfaC*-, and *cfaE*-specific probes are indicated (A, B, C, and E, respectively). Restriction sites defining the ends of these probes and the ends of the DNA fragment used in the in vitro transcription-translation assay are shown. Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; V, *Eco*RV; X, *Xba*I.

0.2. Bacteria used for the β-galactosidase assays were grown in CFA medium. In all other experiments, bacteria were grown in Terrific broth (32). Ampicillin (50 μg/ml) and chloramphenicol (25 μg/ml) were used for selection of plasmid-containing strains.

Recombinant DNA techniques. Plasmid isolation, nucleotide sequence determination, labelling of DNA fragments, and ligations were carried out by standard methods (24). Restriction enzyme fragments were isolated using the Gene Clean kit (Bio 101, Inc., La Jolla, Calif.). Transformation of *E. coli* strains was performed by electroporation with the Gene Pulser (Bio-Rad Laboratories, Richmond, Calif.) according to the manufacturer's directions.

In vitro transcription-translation. To detect proteins encoded by specific DNA fragments, the prokaryotic DNA-directed translation kit (Amersham International, Amersham, England) was used according to the manufacturer's directions.

Northern (RNA) blots. RNA was isolated by using the RNAzol kit (CAMPRO Scientific, Elst, The Netherlands) according to the manufacturer's directions. Electrophoresis of 40 μg of RNA was performed using formaldehyde-agarose gels as described previously (18). RNA marker (Boehringer Mannheim, Mannheim, Germany) was loaded on the gel as a molecular weight marker. RNA was blotted onto N-hybond membrane (Amersham International) with VacuGene (Pharmacia LKB Biotechnology, Uppsala, Sweden) according to the manufacturer's directions. Hybridizations were performed overnight at 42°C in 50% formamide–5× Denhardt's solution–0.1% sodium dodecyl sulfate (SDS)–5× SSPE (1× SSPE is 0.01 M sodium phosphate [pH 7.0], 0.18 M NaCl, and 0.001 M EDTA) containing denatured herring sperm DNA (100 μg/ml). Washings were done in 1× SSPE–0.1% SDS at 42°C. Probes were removed from the filter by washing in 50% formamide–0.1× SSPE–0.05% SDS at 80°C, and the removal of the probe was checked by exposing the filter to a film. In order to visualize the RNA marker, filters were finally hybridized with radioactive cDNA of the RNA marker, washed, and exposed to a new film. Radioactive cDNA was made with random primers in the presence of [α-^{32}P]ATP by using SuperScript RNase H$^-$ reverse transcriptase (BRL Life Technologies, Inc., Gaithersburg, Md.).

Primer extension on mRNA. Primer extension experiments were performed as previously described (22).

β-Galactosidase assay. *E. coli* MC1029 containing plasmid pIVB3-529 with or without pIVB3-105 or plasmid pIVB3-710 and pIVB3-105 was grown overnight at 37°C, washed, and resuspended in phosphate-buffered saline. Samples containing 50 μg of protein per ml were used for the β-galactosidase assay with *o*-nitrophenyl-β-D-galactopyranoside as a substrate. In order to assay the β-galactosidase activity, the development of color (420 nm) was measured at 2, 4, 6, and 8 min after the addition of the substrate. Reactions were stopped by adding 1 1/2 volumes of 1 M Na_2CO_3. Each value given represents the average activity obtained from two independent experiments.

PCR techniques. PCRs were performed in 100 μl of 50 mM KCl–10 mM Tris (pH 8.5)–1.5 mM $MgCl_2$–0.1% Triton X-100–200 μM each deoxynucleoside triphosphate–5 U of *Taq* polymerase, 100 pmol of each primer, and 20 ng of the template. Thirty-five cycles were run, with each cycle consisting of 1 min at 95°C, 2 min at 55°C, and 3 min at 72°C.

RESULTS

Detection of proteins encoded by CFA/I region 1. A 6.2-kb *Eco*RV restriction DNA fragment of CFA/I region 1 (Fig. 1, restriction sites) was used as a template in a transcription-translation assay. The product of the *cfaB* gene is produced in larger amounts than other gene products of CFA/I region 1 (Fig. 2). The 39-kDa protein is probably the product of the *cfaE* gene, whereas the 10-kDa protein has to be a degradation product of a larger protein. In order to investigate how this differential expression of genes in region 1 is regulated, we analyzed the different CFA/I region 1-specific mRNAs.

Analysis of CFA/I region 1-specific mRNAs. RNA was isolated from the CFA/I-producing clinical isolate E7473/0. Northern blots of this RNA were hybridized with *cfaA*-, *cfaB*-, *cfaC*-, and *cfaE*-specific probes. The locations of the probes used are shown in Fig. 1.

RNA fragments of 0.7 and 1.4 kb hybridized with a *cfaA*-specific probe (Fig. 3A); however, the mRNA of 0.7 kb hybridized very poorly. The probe was removed from the blot, and the same blot was hybridized with a *cfaB*-specific probe. In Fig. 3B, it is shown that an mRNA of 0.7 kb hybridized very strongly and an mRNA of 1.4 kb hybridized weakly with this probe. The 0.7-kb mRNA hybridizing with the *cfaA*-specific probe will here be designated *cfaA* RNA, and the 0.7-kb mRNA hybridizing with the *cfaB*-specific probe *cfaB* RNA. The 1.4-kb transcript hybridizing with both probes will be referred to as *cfaAB* RNA.

A Northern blot with RNA of the CFA/I-producing strain E7473/0 was also hybridized with a *cfaC*-specific probe and a *cfaE*-specific probe. No distinct bands reacted with either one of the probes. Faint reactions were seen with both probes. However, these results were not completely reproducible with samples of different isolations of RNA (data not shown).

Mapping of the 5′ end of the *cfaB* RNA by primer extension. RNA of CFA/I-producing strain E7473/0 was used to define the precise 5′ end of the *cfaB* RNA. cDNA synthesis was performed with primer PEB (see Fig. 5). The result of this primer extension experiment is shown in Fig. 4 (lanes 1 and 2). Either nucleotide 1486 or 1487 is the 5′ end of this RNA, which

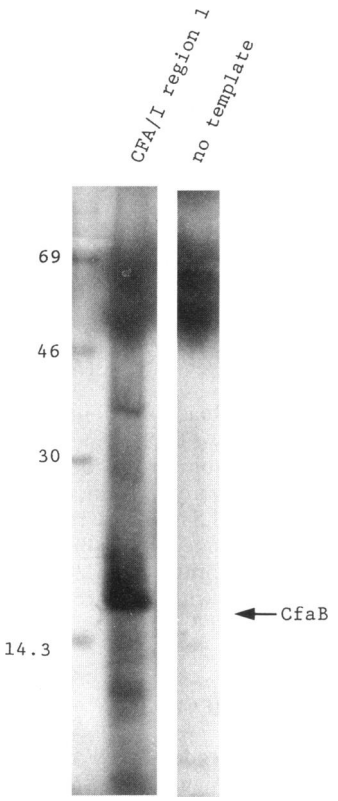

FIG. 2. In vitro expression of CFA/I region 1. Autoradiograph of proteins separated by SDS-polyacrylamide gel electrophoresis. Proteins were produced in an in vitro transcription-translation assay containing a 6.2-kb *Eco*RV restriction DNA fragment (see Fig. 1) of CFA/I region 1 as a template. An experiment in which no DNA template was added is shown as a negative control. The sizes of the molecular mass markers are indicated in kilodaltons.

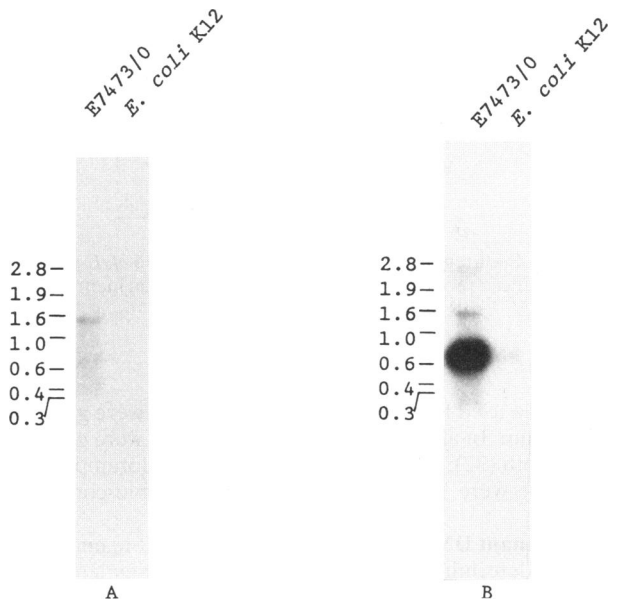

FIG. 3. Autoradiograph of a Northern blot hybridized with a *cfaA*-specific probe (A) or a *cfaB*-specific probe (B). RNA was isolated from the CFA/I-producing clinical isolate E7473/0 and from an *E. coli* K-12 strain as a negative control. The size of the RNA standards used is indicated in kilobases.

indicates that the 5′ end of the *cfaB* RNA is within the sequence of the *cfaA* gene (Fig. 5).

There is no promoter activity directly upstream of the 5′ end of the *cfaB* RNA. A 260-bp fragment containing the DNA upstream of the 5′ end of the *cfaB* RNA was generated by PCR, using oligonucleotides B1 and B2 (Fig. 5). A *Bam*HI and a *Sal*I restriction endonuclease site were introduced at the 5′ end of oligonucleotides B1 and B2, respectively. The PCR fragment was digested with *Bam*HI and *Sal*I and ligated in front of the promoterless *lacZ* gene, resulting in plasmid pIVB3-529. The nucleotide sequence of the insert was determined.

Two deletions compared with the sequence published previously (11) were present in the construct pIVB3-529. The nucleotide sequence of the original DNA was rechecked and was shown to contain the same deletions. The sequence in the GenBank data base under the accession number M55661 has been corrected according to these findings.

No significant β-galactosidase activity was observed in a strain containing the plasmid pIVB3-529 (Fig. 6, +). The introduction of pIVB3-105, encoding the positive regulator CfaD of CFA/I region 2, did not elevate this activity (Fig. 6, △). A strain containing construct pIVB3-710, the promoter upstream of *cfaA* cloned in front of *lacZ* (12), and pIVB3-105, encoding CfaD (25), was used as a positive control (Fig. 6, ○).

These results indicate that the *cfaB* RNA is generated by processing of the *cfaAB* RNA.

RNA processing of the *cfaAB* RNA by defined RNA endoribonucleases. Both CFA/I region 1 (plasmid NTP513 [33]) and CFA/I region 2 (plasmid pIVB3-100 [25]) were introduced into RNase E-, RNase P-, and RNase III-deficient strains and their respective wild-type isogens.

The RNase III mutant strain BL321 (31) and its wild-type isogen BL322 containing both region 1 and region 2 did produce *cfaB*-specific mRNA. No accumulation of the *cfaAB* RNA could be observed with either a *cfaB*-specific probe or a *cfaA*-specific probe (data not shown). This indicates that RNase III is not involved in the processing of the *cfaAB* transcripts.

In contrast to RNase III mutant strains, RNase E and RNase P mutant strains have severe growth deficiencies. Strain N3431 contains the temperature-sensitive mutation *rne-3071* (1). At 44°C, this strain is RNase E-deficient and thereby unable to grow. Strain N3431 and its wild-type isogen, N3433, harboring both region 1 and region 2 were grown at 42 and 37°C. Both strain N3431 and N3433, containing the appropriate plasmids, produced *cfaB* RNA at 37 and 42°C. No *cfaAB* transcripts could be detected in strain N3431 at 42°C with a *cfaB*- or a *cfaA*-specific probe (data not shown), indicating that RNase E is probably not involved in the processing of *cfaAB* RNA. We cannot rule out the possibility that the small amount of RNase E present in strain N3431 grown at 42°C is processing the *cfaAB* transcripts in these experiments.

Similar experiments were performed with the RNase P-deficient strain NHY312 and its wild-type isogen, NHY322 (16). No accumulation of *cfaAB* RNA could be observed in the RNase P mutant.

DISCUSSION

We demonstrate here that the amount of the different mRNA fragments of CFA/I region 1 involves processing of RNA and differential decay of the processed RNAs. The

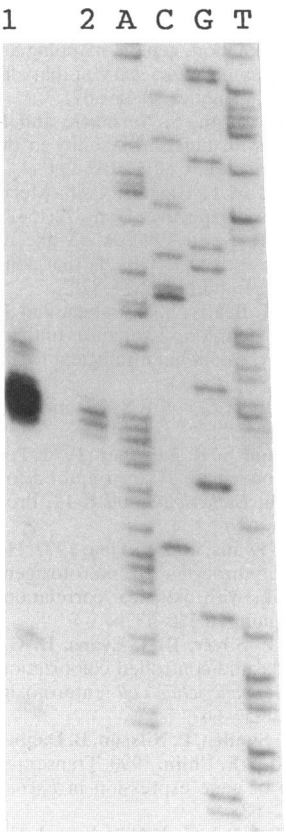

FIG. 4. Primer extension analysis of RNA isolated from the CFA/I-producing clinical isolate E7473/0. End-labelled primer PEB (see Fig. 5) was used to prime cDNA synthesis. Products were analyzed on a 4.5% polyacrylamide sequencing gel together with the products of a dideoxy sequencing reaction with end-labelled primer PEB, with plasmid NTP513 (CFA/I region 1) as a template. Lanes: 1 and 2, primer extension with 200 and 20 ng, respectively, of primer PEB.

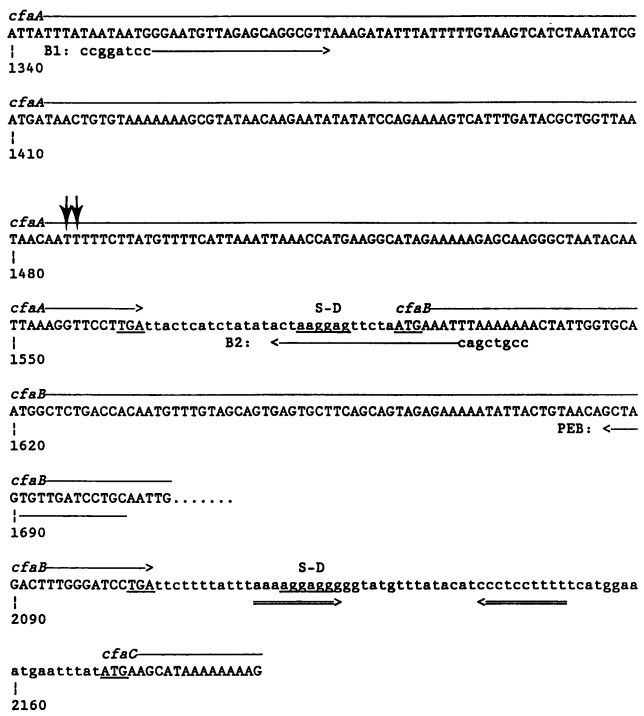

FIG. 5. Nucleotide sequence of regions involved in the processing and differential decay of RNA transcribed from CFA/I region 1. Stop codons, start codons, and ribosome-binding sites are underlined. Oligonucleotides B1, B2, and PEB are indicated underneath the sequence. The two start points of the *cfaB* mRNA are indicated by vertical arrows, and the stem-loop structure is shown by double-lined horizontal arrows.

primary *cfaAB* transcript is processed into a stable *cfaB* mRNA and an unstable *cfaA* mRNA. The stability of the *cfaB* RNA is consistent with the presence of a stem-loop structure directly downstream of the *cfaB* gene (Fig. 5). Stem-loop structures are known to stabilize upstream RNA by blocking the activity of PNPase and RNase II, the two 3′ to 5′ exoribonucleases in *E. coli* (3). A schematic model of the processing of *cfaAB* RNA is given in Fig. 7. Since no distinct *cfaC*- or *cfaE*-specific RNA can be detected, the mRNAs of these genes are probably degraded very rapidly and/or produced in small amounts. The fact that *cfaC*-and *cfaE*-specific RNAs are produced in small amounts is probably due to the fact that the stem-loop structure downstream of the *cfaB* gene acts as an imperfect terminator of transcription.

Most likely, the RNA processing and degradation events described above regulate the differential expression of the genes in CFA/I region 1. In vitro, the *cfaB* gene product is produced in larger amounts than other gene products of region 1. In vivo, the product of the *cfaB* mRNA, the CFA/I subunit protein, is probably needed in excess of the other gene products of region 1. The other gene products can either be minor subunit proteins or play a role in the assembly of subunit proteins into fimbriae.

We have suggested earlier that the mRNA reacting with a *cfaB*-specific probe is a *cfaAB* transcript of approximately 1.2 kb (12). We show here that the major RNA reacting with a *cfaB*-specific probe is a *cfaB* mRNA of approximately 0.7 kb (Fig. 3B). The misinterpretation of our previous results is probably due to two facts. First, in the experiments described

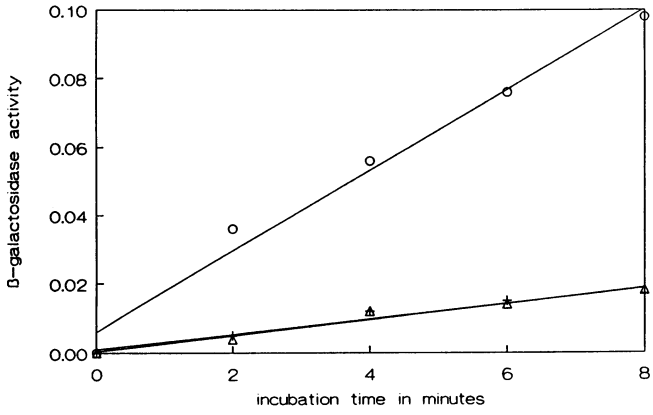

FIG. 6. β-Galactosidase activity of construct pIVB3-529 (+) containing a 260-bp fragment upstream of the *cfaB* gene cloned in front of the *lacZ* gene. Introduction of pIVB3-105 encoding the positive regulator, CfaD, of CFA/I did not elevate the activity (△). A strain containing both construct pIVB3-710 (which has the promoter upstream of *cfaA* cloned in front of *lacZ*) and construct pIVB3-105 (encoding CfaD) was used as a positive control (○).

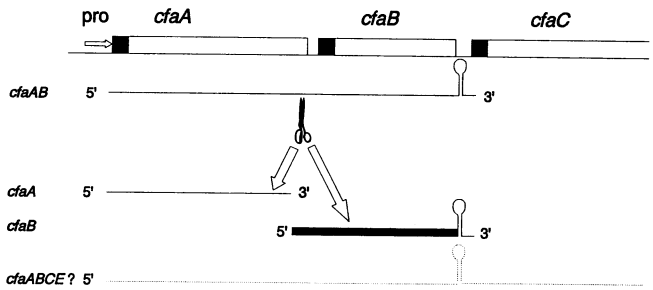

FIG. 7. Schematic presentation of the CFA/I region 1-specific RNA fragments present in CFA/I-producing *E. coli* strains. The *cfaA* and the *cfaB* genes and a part of the *cfaC* gene are indicated. Underneath the DNA, the *cfaAB*, *cfaA*, and *cfaB* mRNAs as well as the putative *cfaABCE* mRNA are given. The scissors indicate the position of the site-specific cleavage of the RNA by an endoribonuclease. The stem-loop structure involved in stabilization of the *cfaB* RNA is depicted.

in our previous report we denatured the RNA insufficiently prior to loading it on the formaldehyde-agarose gel. Second, in these experiments the RNA was not hybridized with a *cfaA*-specific probe. If this had been done, it would have been obvious that the mRNA reacting strongly with a *cfaB*-specific probe does not contain *cfaA* sequences.

An abundant *cfaB* mRNA and small amounts of the *cfaA* and *cfaAB* mRNAs could also be explained by an alternative model in which two promoters are present. The first promoter in front of the *cfaA* gene should then generate the *cfaA* and *cfaAB* mRNAs, and a second promoter in front of the *cfaB* gene should generate the *cfaB* mRNA. We already confirmed the presence of a promoter in front of the *cfaA* gene (25). No promoter activity could be detected in front of the *cfaB* gene (Fig. 6) in contrast with the data of Karjalainen et al. (15). Two additional facts are in agreement with our experimental results. (i) No consensus sequence for an *E. coli* promoter is present directly upstream of the start of the cfaB mRNA, and (ii) a putative promoter in front of the *cfaB* mRNA would be inside the coding region of the *cfaA* gene. Therefore, we conclude that no promoter is present directly upstream of the *cfaB* gene. With the data described here, we cannot explain the results of Karjalainen et al. (15).

Accumulation of the *cfaAB* RNA could not be observed in RNase III-, RNase E-, or RNase P-deficient strains, indicating that these endoribonucleases are probably not involved in the cleavage of the *cfaAB* transcript. Recently, additional *E. coli* endoribonucleases which may be involved in the processing of the *cfaAB* transcript have been described (19, 30).

Differential stability of mRNA segments of polycistronic transcripts has been shown to regulate the level of gene expression within several bacterial operons (for examples, see references 2, 5, 20, and 21). Here, we show the existence of processing of RNA transcribed from CFA/I region 1. Probably, these processing events regulate the differential expression of genes in CFA/I region 1.

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REFERENCES

1. **Apirion, D.** 1978. Isolation, genetic mapping and some characterization of a mutation in *Escherichia coli* that affects the processing of ribonucleic acid. Genetics **90:**659–671.
2. **Båga, M., M. Göransson, S. Normark, and B. E. Uhlin.** 1988. Processed mRNA with different stability in the regulation of *E. coli* pilin gene expression. Cell **52:**197–206.
3. **Belasco, J. G., and C. F. Higgins.** 1988. Mechanisms of mRNA decay in bacteria: a perspective. Gene **72:**15–23.
4. **Caron, J., and J. R. Scott.** 1990. A *rns*-like regulatory gene for colonization factor antigen I (CFA/I) that controls expression of CFA/I pilin. Infect. Immun. **58:**874–878.
5. **Chen, C.-Y. A., J. T. Beatty, S. N. Cohen, and J. G. Belasco.** 1988. An intercistronic stem-loop structure functions as an mRNA decay terminator necessary but insufficient for *puf* mRNA stability. Cell **52:**609–619.
6. **Deutscher, M. P.** 1985. *E. coli* RNases: making sense of alphabet soup. Cell **40:**731–732.
7. **Donovan, W. P., and S. R. Kushner.** 1986. Polynucleotide phosphorylase and ribonuclease II are required for cell viability and mRNA turnover in *Escherichia coli* K-12. Proc. Natl. Acad. Sci. USA **83:**120–124.
8. **Evans, D. G., D. J. Evans, and W. Tjoa.** 1977. Hemagglutination of human group A erythrocytes by enterotoxigenic *Escherichia coli* isolated from adults with diarrhea: correlation with colonization factor. Infect. Immun. **18:**330–337.
9. **Evans, D. G., R. P. Silver, D. J. Evans, D. G. Chase, and S. L. Gorbach.** 1975. Plasmid-controlled colonization factor associated with virulence in *Escherichia coli* enterotoxigenic for humans. Infect. Immun. **12:**656–667.
10. **Göransson, M., B. Sondén, P. Nilsson, B. Dagberg, K. Forsman, K. Emanuelsson, and B. E. Uhlin.** 1990. Transcriptional silencing and thermoregulation of gene expression in *Escherichia coli*. Nature (London) **344:**682–685.
11. **Hamers, A. M., H. J. Pel, G. A. Willshaw, J. G. Kusters, B. A. M. van der Zeijst, and W. Gaastra.** 1989. The nucleotide sequence of the first two genes of the CFA/I fimbrial operon of human enterotoxigenic *Escherichia coli*. Microb. Pathog. **6:**297–309.
12. **Jordi, B. J. A. M., B. Dagberg, L. A. M. de Haan, A. M. Hamers, B. A. M. van der Zeijst, W. Gaastra, and B. E. Uhlin.** 1992. The positive regulator CfaD overcomes the repression mediated by histone-like protein H-NS (H1) in the CFA/I fimbrial operon of *Escherichia coli*. EMBO J. **11:**2627–2632.
13. **Jordi, B. J. A. M., A. H. M. van Vliet, G. A. Willshaw, B. A. M. van der Zeijst, and W. Gaastra.** 1991. Analysis of the first two genes of the CS1 fimbrial operon in human enterotoxigenic *Escherichia coli* of serotype O139:H28. FEMS Microbiol. Lett. **80:**265–270.
14. **Jordi, B. J. A. M., G. A. Willshaw, B. A. M. van der Zeijst, and W. Gaastra.** 1992. The complete nucleotide sequence of region 1 of the CFA/I fimbrial operon of human enterotoxigenic *Escherichia coli*. DNA Sequence **2:**257–263.
15. **Karjalainen, T. K., D. G. Evans, D. J. Evans, Jr., D. Y. Graham, and C.-H. Lee.** 1991. Iron represses the expression of CFA/I fimbriae of enterotoxigenic *E. coli*. Microb. Pathog. **11:**317–323.
16. **Kirsebom, L. A., M. F. Baer, and S. Altman.** 1988. Differential effects of mutations in the protein and RNA moieties of RNase P on the efficiency of suppression by various tRNA suppressors. J. Mol. Biol. **204:**879–888.
17. **Krogfelt, K. A.** 1991. Bacterial adhesion: genetics, biogenesis, and role in pathogenesis of fimbrial adhesins of *Escherichia coli*. Rev. Infect. Dis. **13:**721–735.
18. **Lehrach, H., D. Diamond, J. M. Wozney, and H. Boedtker.** 1977. RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. Biochemistry **16:**4743–4751.
19. **Lundberg, U., A. von Gabain, and Ö. Melefors.** 1990. Cleavages of the *ompA* and *bla* mRNA control stability: studies with an *E. coli* mutant altering mRNA stability and a novel endoribonuclease. EMBO J. **9:**2731–2741.
20. **Newbury, S. F., N. H. Smith, and C. F. Higgins.** 1987. Differential mRNA stability controls relative gene expression within a polycistronic operon. Cell **51:**1131–1143.
21. **Nilsson, P., and B. E. Uhlin.** 1991. Differential decay of a

polycistronic *Escherichia coli* transcript is initiated by RNaseE-dependent endonucleolytic processing. Mol. Microbiol. **5:**1791–1799.

22. **Nuijten, P. J. M., F. J. A. M. van Asten, W. Gaastra, and B. A. M. van der Zeijst.** 1990. Structural and functional analysis of 2 *Campylobacter jejuni* flagellin genes. J. Biol. Chem. **265:**17798–17804.
23. **Sack, R. B.** 1975. Human diarrhoeal disease caused by enterotoxigenic *Escherichia coli*. Annu. Rev. Microbiol. **29:**333–353.
24. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Savelkoul, P. H. M., G. A. Willshaw, M. M. McConnell, H. R. Smith, A. M. Hamers, B. A. M. van der Zeijst, and W. Gaastra.** 1990. Expression of CFA/I fimbriae is positively regulated. Microb. Pathog. **8:**91–99.
26. **Schneider, K., and C. F. Beck.** 1986. Promoter-probe vector for the analysis of divergently arranged promoters. Gene **42:**37–48.
27. **Scott, J. R., J. C. Wakefield, P. W. Russell, P. E. Orndorff, and B. J. Froehlich.** 1992. CooB is required for assembly but not transport of CS1 pilin. Mol. Microbiol. **6:**293–300.
28. **Smith, H. R., A. Cravioto, G. A. Willshaw, M. M. McConnell, S. M. Scotland, R. J. Gross, and B. Rowe.** 1979. A plasmid coding for the production of colonization factor antigen I and heat stable enterotoxin in strains of *Escherichia coli* of serogroup O78. FEMS Microbiol. Lett. **6:**255–260.
29. **Smith, H. R., G. A. Willshaw, and B. Rowe.** 1982. Mapping of a plasmid, coding for colonization factor antigen I and heat-stable enterotoxin production, isolated from an enterotoxigenic strain of *Escherichia coli*. J. Bacteriol. **149:**264–275.
30. **Srivastava, S. K., V. J. Cannistraro, and D. Kennell.** 1992. Broad-specificity endoribonucleases and mRNA degradation in *Escherichia coli*. J. Bacteriol. **174:**56–62.
31. **Studier, F. W.** 1975. Genetic mapping of a mutation that causes ribonuclease III deficiency in *Escherichia coli*. J. Bacteriol. **124:**307–316.
32. **Tartof, K. D., and C. A. Hobbs.** 1987. Improved media for growing plasmid and cosmid clones. Bethesda Res. Lab. Focus **9:**12.
33. **Willshaw, G. A., H. R. Smith, and B. Rowe.** 1983. Cloning of regions encoding colonisation factor antigen 1 and heat-stable enterotoxin in *Escherichia coli*. FEMS Microbiol. Lett. **16:**101–106.