

tRNA^{Arg} (*fimU*) and Expression of SEF14 and SEF21 in *Salmonella enteritidis*

S. C. CLOUTHIER,^{1,2} S. K. COLLINSON,¹ A. P. WHITE,¹ P. A. BANSER,¹ AND W. W. KAY^{1,2*}

Department of Biochemistry and Microbiology, University of Victoria, Victoria, British Columbia V8W 3P6,¹
and Microtek International Ltd., Saanichton, British Columbia V8X 3X1,² Canada

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A Tn10 insertion affecting SEF14 fimbrial synthesis in *Salmonella enteritidis* was located 13 bp upstream of a gene designated *fimU*. The 77-bp DNA sequence of *fimU* from *S. enteritidis* was identical to that of *fimU* encoding tRNA^{Arg} (UCU) from *Salmonella typhimurium* and 96% identical to that of the *Escherichia coli argU* homolog. Furthermore, the open reading frame adjacent to and overlapping the 3' end of *fimU* was similar to the prophage DLP12 integrase gene. The *fimU*-encoded transcript comigrated with total cellular tRNA and was predicted to form a tRNA-like cloverleaf structure containing the arginine anticodon UCU. Thus, *fimU* encoded a tRNA^{Arg} specific for the rare codon AGA. *fimU* mapped to the SEF21 *fim* operon located 15 C's from the *sef14* gene cluster. Although *fimU* was located within the SEF21 *fim* gene cluster, the *fimU* Tn10 insertion mutant of *S. enteritidis* was found to be defective in SEF14 as well as SEF21 (type 1) fimbria production. SEF17 and SEF18 fimbria production was not affected. Complementation of this mutant with plasmid-borne *fimU* restored normal production of the fimbrins SefA and FimA as well as their respective fimbriae SEF14 and SEF21. This is the first description of tRNA simultaneously controlling the production of two distinct fimbriae.

Regulation of fimbria biosynthesis in bacteria is multifactorial and complex. In *Escherichia coli*, the expression of type 1 fimbriae is transcriptionally regulated in part by an inversion-dependent, phase-variable mechanism that involves two site-specific recombinases (17, 24, 27) and a tRNA^{Leu} molecule (32). tRNA^{Leu}, specific for the rare leucine codon UUG, stimulates type 1 fimbria synthesis by influencing the switch from phase off to phase on (35). Recently, type 1 fimbria expression in *Salmonella typhimurium* has been shown to be regulated by mechanisms that are different from those controlling type 1 fimbria expression in *E. coli* (41). However, a common regulatory theme does exist in that a tRNA, specific for the rare arginine codons AGA and AGG, is required (40). Swenson et al. (40) suggest that the amount of tRNA^{Arg} (UCU) available in *S. typhimurium* may influence the expression of three genes encoding regulatory proteins of the *fim* gene cluster, since in each of these genes there is a high frequency of rare AGA codons recognized by tRNA^{Arg} (UCU).

Salmonella enteritidis 27655-3b produces at least four fimbrial types: SEF17 (10), SEF18 (6), SEF21 (type 1 fimbriae) (30), and SEF14 (7, 14). Although little is known about how the expression of the operons is regulated, SEF21 and SEF14 fimbriae are produced under similar environmental conditions (5, 12). Thus, the question arises as to whether or not their expression is coregulated. In a previous study, a Tn10 insertion mutant, *S. enteritidis* 3b-122, was generated which no longer produced SEF14 fimbriae and carried the transposon outside of *sef4*, the structural gene for these fimbriae (14). Further characterization of 3b-122 in this study indicated that this mutant was also defective in type 1 fimbria (SEF21) production, suggesting that the Tn10 interrupted a gene whose product coregulated the expression of both SEF14 and SEF21 fimbriae. The results of this study show for the first time that the production of two fimbriae is coregulated by the same tRNA.

* Corresponding author. Mailing address: Department of Biochemistry and Microbiology, University of Victoria, Victoria, B.C. V8W 3P6, Canada. Phone: (250) 721 7078. Fax: (250) 721 8882. E-mail: wkay@uvic.ca.

MATERIALS AND METHODS

Bacterial strains, plasmids, media, and growth conditions. *S. enteritidis* 27655-3b, originally isolated from human feces, was provided by T. Wadstrom (University of Lund, Lund, Sweden). *S. enteritidis* 27655-3b-122, a Tn10 insertion mutant of the parent strain, was constructed by Feutrier et al. (14). *E. coli* DH5 α and *S. enteritidis* 3b-122 were used as hosts for pSFA (11), pLU/TA 4-1, and pGEM-T1. To create pLU/TA 4-1, PCR-amplified *fimU* was cloned into pGEM-T (Promega Corp.), a TA cloning vector containing 3'-terminal thymidines. To create pGEM-T1, the 3'-overhanging thymidines of pGEM-T were filled in with dATP and T4 DNA polymerase prior to ligation (38).

Bacteria were grown at 37°C with shaking in Luria-Bertani (LB) broth (36) supplemented with ampicillin to a final concentration of 250 μ g/ml except where noted. To analyze the production of fimbriae by *S. enteritidis*, the cells were grown in various liquid media under different growth conditions (Table 1). Cultures grown in LB broth and terrific broth (TFB) (38) were transferred to ice 24 h after inoculation, whereas cultures grown in colonization factor antigen (CFA) medium (13) and T broth (10) were transferred to ice 48 h after inoculation. All the cultures were standardized to an optical density at 630 nm (OD₆₃₀) of 1.

Subcloning Tn10 from *S. enteritidis* 27655 3b-122. *S. enteritidis* 3b-122 chromosomal DNA was isolated by the method of Alm et al. (1), purified by CsCl centrifugation (38), and digested with *Hind*III. To subclone the Tn10-containing chromosomal DNA fragment, size fractionated *Hind*III fragments (2 to 3 and 3 to 5 kb) were purified from an agarose gel with Sephaglas (Pharmacia Biotech), ligated to *Hind*III-digested and -dephosphorylated cloning vector pTZ19R, and then introduced into *E. coli* DH5 α by transformation (38). A total of 2,880 colonies grown on Hybond N⁺ membranes (Amersham) were screened by hybridization to the oligonucleotide probe Tn10 IS10L+R (5' GCAGAATTGGT AAAGAGA 3'). This probe, complementary to the sequence located 134 bp inside the insertion sequence of Tn10, was used to identify Tn10-containing clones. The probe, end labelled with [γ -³²P]ATP, was hybridized to the membranes at 45°C in prehybridization buffer (38) containing 200 μ g of herring sperm DNA (Sigma)/ml. Following hybridization, the membranes were washed in 0.2 \times SSC buffer (1 \times SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 0.1% sodium dodecyl sulfate (SDS) at 45°C, and the results were recorded by autoradiography on Kodak BioMax film.

DNA sequencing and computer analyses. The Tn10-positive clones and the three *fimU* PCR products amplified with primers located outside the *fimU* gene were sequenced with Sequenase version 2 (United States Biochemicals). The custom oligonucleotide primer Tn10IS10L+R was synthesized on a PCR-MATE EP model 391 DNA synthesizer (Applied Biosystems Inc.). The DNA sequences obtained were analyzed with DNA Strider 1.1 (26). Similarity searches of the National Center for Biotechnology Information (NCBI) databases were conducted with the program BLASTN (2).

PCR amplification of *fimU*. Custom oligonucleotide primers *fimULT* (TAAT AGCGATACGAGAATTCAAATAATCCTACCGCAGG) and *fimULB* (CAGATATGCTCACCTAAGCTTAAATCATTTAACGGAACACGG) were designed based on the *S. typhimurium* chromosomal DNA sequence flanking

TABLE 1. Production of SefA, FimA, AgfA, and SefD fimbriae by *S. enteritidis* 3b and *S. enteritidis* 3b-122

Medium ^a	°C	rpm	Fimbrin production ^b							
			<i>S. enteritidis</i> 3b				<i>S. enteritidis</i> 3b-122			
			SefA	FimA	AgfA	SefD	SefA	FimA	AgfA	SefD
TFB	28	0	—	+	—	+++	—	—	—	+++
TFB		250	—	++	±	+++	—	—	—	+++
TFB	37	0	+	++	—	+++	—	—	—	+++
TFB		250	— ^c	++	±	+++	—	—	+	+++
LB	28	0	—	++	++	+++	—	—	—	+++
LB		250	—	++	+++	+++	—	—	+++	+++
LB	37	0	+++	+++	++	+++	—	—	—	+++
LB		250	++	++	+++	+++	—	—	+	+++
CFA	28	0	—	++	+++	+++	—	±	+++	+++
CFA		250	—	+	+++	+++	—	±	+++	+++
CFA	37	0	+++	++	+++	+++	—	±	+++	+++
CFA		250	— ^c	+	+++	+++	—	±	+++	+++
T	28	0	—	++	+++	+++	—	—	+++	+++
T		250	—	+	+++	+++	—	—	+++	+++
T	37	0	+	++	+++	+++	—	—	+++	+++
T		250	— ^c	+	+++	+++	—	—	+++	+++

^a All cultures were grown in liquid medium for 24 (TFB and LB) or 48 h (T and CFA).

^b The intensities of fimbriae bands detected on Western blots are indicated as follows: —, none detected; ±, very faint; +, weak; ++, moderate; +++, strong.

^c SefA was not detected on four of six Western blots but a very faint SefA band was detected on two of six Western blots.

fimU and were synthesized by Gibco BRL. *fimU* was PCR amplified from a previously prepared cosmid clone, pPB523 (12), with *fimULT* and *fimULB*. To facilitate the cloning of the amplified product, the primers were designed to contain an *EcoRI* site and a *HindIII* site, respectively (underlined). Amplification was carried out in a 100- μ l reaction volume containing 10 μ l of pPB523 (0.01 μ g/ml), 25 pmol of each primer, the four deoxynucleotide triphosphates (Boehringer Mannheim) at 0.5 mM each, and 2 U of *Taq* DNA polymerase (Boehringer Mannheim) in reaction buffer consisting of 50 mM Tris-HCl (pH 8.5), 20 mM KCl, 2.5 mM MgCl₂, and 0.5 mg of bovine serum albumin/ml. The *Taq* enzyme was added after an initial 3-min denaturation step at 95°C (4). Thermocycling was performed in a PTC-100TM Programmable Thermal Controller (MJ Research Inc.) as follows: 1 cycle of 75°C, 1 min; 50°C, 2 min; 74°C, 2 min and 30 cycles of 95°C, 1 min; 50°C, 1 min; 74°C, 2 min, followed by an 8-min elongation at 74°C.

Subcloning PCR-amplified *fimU*. PCR-amplified *fimU* was purified from a 1% agarose gel with Sephaglas, ligated to pGEM-T according to the manufacturer's instructions (Promega Corp.), and then transformed into *E. coli* DH5 α (38).

Mapping of *fimU* on genomic restriction maps of *Salmonella* and *E. coli* strains. The *fimU* gene was mapped as previously described for the four fimbriae genes *sefA*, *agfA*, *fimA*, and *sefD* (8). The *fimU* probe, prepared by running *EcoRI*- and *HindIII*-digested pLU/TA 4-1 on a 1% agarose gel and purifying the fragment with Sephaglas, was labelled with [α -³²P]dATP (Pharmacia Biotech) by nick translation. The radiolabelled *fimU* probe was hybridized to nitrocellulose blots containing *XbaI*- and *BlnI*-digested *E. coli*, *S. typhimurium*, and *S. enteritidis* genomic DNA separated by pulsed field gel electrophoresis (blots were provided by K. Sanderson and S.-L. Liu; see reference 8).

RNA extraction and Northern blot analysis. Total RNA was prepared from *S. enteritidis* 3b, 3b-122, 3b-122 pGEM-T1, and 3b-122 pLU/TA 4-1 grown statically in LB or CFA broth at 37°C for 45 h by a modification of the procedure of McCormick et al. (28) as described in Clouthier et al. (7). For *fimU* transcript analysis, the RNA was separated on a 10% polyacrylamide gel containing 8 M urea and transferred onto Hybond N⁺ membranes (Amersham) with transfer buffer (0.025 M phosphate buffer [pH 6.5]) and an LKB Pharmacia semidry blotting apparatus. For *sefA* transcript analysis, the electrophoretic separation of total cellular RNA and its subsequent transfer to Hybond N⁺ membranes (Amersham) were performed as described in Fourney et al. (15). The *fimU*- and *sefA*-specific probes used for Northern blot analysis were gel purified from *EcoRI* and *HindIII* digests of pLU/TA 4-1 and pSFA, respectively, with Sephaglas. The probes were labelled with [α -³²P]dATP (Pharmacia Biotech) by nick translation and hybridized to the blots at 65°C for 18 h in the presence of 200 μ g of herring sperm DNA (Sigma)/ml. The membranes were washed at high stringency (0.2 \times SSC buffer-0.1% SDS, 65°C), and the results were recorded on Kodak BioMax or X-Omat AR5 film.

SDS-PAGE and Western blot analysis. Whole-cell lysates of *S. enteritidis* 3b or clones of this strain were screened for the presence of four fimbrial types. SEF14, 18, and 21 were solubilized from whole cells with SDS-polyacrylamide gel electrophoresis (PAGE) sample buffer supplemented with 0.2 M glycine (pH 2,

100°C, 10 min), whereas SEF17 fimbriae were solubilized from whole cells with formic acid according to the method of Collinson et al. (9, 10). A portion of each culture (1 OD₆₃₀ unit) was resuspended in 200 μ l of sample buffer, and 10 μ l (0.01 OD₆₃₀ unit) was loaded per lane. Proteins in these samples were separated by SDS-PAGE, electrophoretically transferred to nitrocellulose, and screened with rabbit polyclonal anti-SEF14 (7), SEF17 (10), SEF18 (6), or SEF21 immune serum (30). Immunoreactive proteins were detected with goat anti-rabbit immunoglobulin G-alkaline phosphatase conjugates (Cedarlane) and visualized with 5-bromo-4-chloro-3-indolylphosphate and Nitro Blue Tetrazolium (Sigma).

Electron microscopy. SEF14 and SEF21 fimbriae on *S. enteritidis* 3b, 3b-122, 3b-122 pGEM-T1, and 3b-122 pLU/TA 4-1 were immunogold labelled with SEF14- or SEF21-specific rabbit polyclonal immune sera followed by incubation with protein A-15-nm-diameter gold particles (Cedarlane). Negative staining was performed as described previously (10).

Nucleotide sequence accession number. The nucleotide sequence reported herein for *fimU* has been submitted to GenBank and has been given the accession number AF013136.

RESULTS

Fimbria production in *S. enteritidis* 3b-122. Production of SEF14, -17, -18, and -21 fimbriae by the *S. enteritidis* Tn10 mutant 3b-122 grown under various growth conditions was assessed by Western blotting with fimbria-specific antisera, and the results were compared to those obtained with the wild-type strain *S. enteritidis* 3b. The Tn10 mutation in 3b-122 had a pronounced effect on SEF14 and SEF21 production but little or no effect on SEF17 and SEF18 production (Table 1). As previously reported, SEF14 fimbriae were not expressed by 3b-122 grown in static CFA broth at 37°C (Table 1). Further characterization in this study, however, showed that 3b-122 lost SEF14 expression under all the growth conditions in which 3b was SEF14 positive (Table 1). In addition to the SEF14-negative phenotype, 3b-122 was also defective for type 1 fimbria (SEF21) production. The wild-type strain produced FimA under all growth conditions tested, whereas 3b-122 only produced FimA in CFA broth cultures. Thus, the result of the Tn10 insertion was the complete loss of SEF14 expression under all growth conditions and selective loss of SEF21 expression under certain growth conditions. The altered production of SEF14 and SEF21 fimbriae in the Tn10 insertion mutant rel-

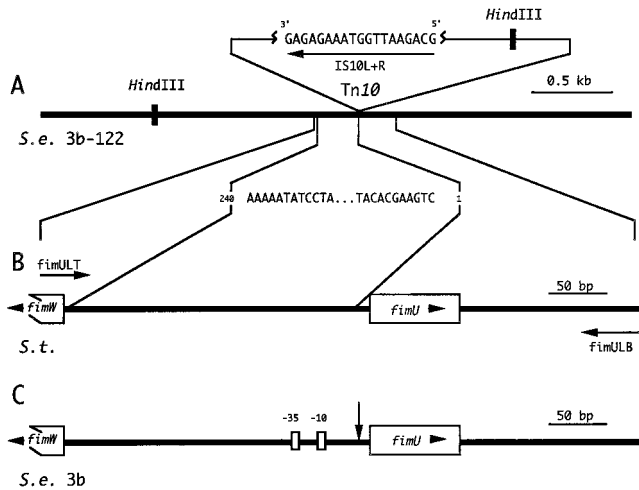


FIG. 1. Location of *Tn10* on the *S. enteritidis* 3b-122 chromosome and identification of the genes flanking the *Tn10* insertion in *S. enteritidis* 3b. (A) Schematic diagram of *S. enteritidis* (*S.e.*) 3b-122 chromosomal DNA (black line) showing the *Tn10* insert and the strategy used to obtain the chromosomal DNA sequence adjacent to one side of this insert. A 3-kb *HindIII* fragment comprising 3b-122 chromosomal DNA fused to one end of *Tn10* was identified by hybridization with the *Tn10* oligonucleotide IS10L+R. IS10L+R was also used as a sequencing primer to obtain 240 bp of DNA sequence from the subcloned *HindIII* fragment. (B) Schematic diagram of the *S. typhimurium* (*S.t.*) chromosome (black line) between *fimW* and *fimU* of the type I fimbrial gene cluster that was homologous to the 240 bp of *S. enteritidis* 3b-122 DNA sequence. Two 42-bp oligonucleotide primers, *fimULT* and *fimULB* (horizontal arrows), were made based on the *S. typhimurium* sequence previously deposited in GenBank (L19338) by Swenson and Clegg (39). (C) Segment of the *S. enteritidis* 3b chromosome (black line) amplified by PCR with the primers *fimULT* and *fimULB*. This amplified DNA segment was subcloned and sequenced (Fig. 2) to identify the DNA flanking the *Tn10* insert. The *Tn10* insertion point (vertical arrow) was determined to be between the -10 region and the start of the *fimU* gene. The presence of *fimW*, *fimU*, and the -35 region on the 3b chromosome is also noted.

ative to the wild-type expression patterns suggested that the transposon insertion interrupted a gene whose product was required for both SEF14 and SEF21 fimbria expression.

Identification of the *Tn10* insertion site in *S. enteritidis* 3b-122. To determine the *Tn10* insertion site, *HindIII* fragments of 3b-122 chromosomal DNA were subcloned into pTZ19. Clones containing *Tn10* were identified with the probe IS10L+R, which hybridized within the insertion sequence located at either end of the transposon (Fig. 1A). Of the 17 *Tn10*-positive clones identified, 3 were subjected to DNA sequence analysis. Comparison of the 3b-122 DNA sequence flanking *Tn10* to sequences listed in the NCBI databases revealed that the sequence was 99% identical to that of the region located between *fimW* and *fimU* of the *S. typhimurium* type 1 fimbrial gene cluster. Thus, on the basis of DNA sequence comparison, *Tn10* was inserted 13 bp upstream of the predicted start site of the gene, which will hereafter be referred to as *fimU* (Fig. 1A and B).

DNA sequence analysis of *fimU* subcloned from *S. enteritidis* 3b. By using primers *fimULT* and *fimULB* designed from the sequence flanking the *fimU* gene in *S. typhimurium*, a 490-bp fragment was PCR amplified from the cosmid clone pPB523-G containing 35 kb of *S. enteritidis* 3b DNA (Fig. 1B). The *fimU* PCR product was subcloned into vector pGEM-T (Fig. 1B). Nucleotide sequence analysis of three clones revealed a potential promoter, but a putative translated protein could not be detected by open reading frame analysis. Comparison of the DNA sequence downstream of the potential promoter to se-

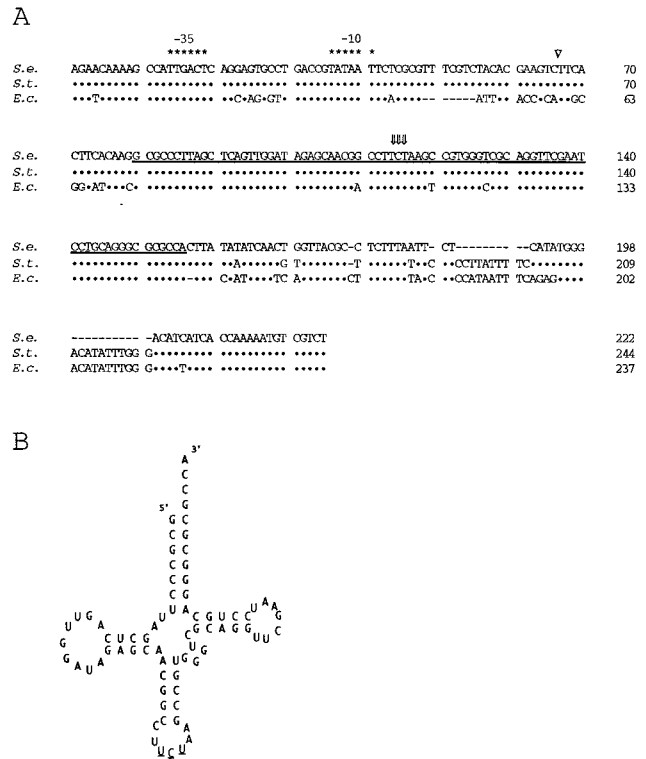


FIG. 2. Sequence comparison of *fimU* from *S. enteritidis* 3b (*S.e.*) with *fimU* of *S. typhimurium* (*S.t.*) and *argU* of *E. coli* (*E.c.*) as well as the predicted *fimU* RNA secondary structure. (A) Alignment of *S. enteritidis* *fimU* DNA sequence with both the *S. typhimurium* *fimU* (39) and *E. coli* (31) *argU* gene sequences. Symbols: ●, DNA sequence identity; —, gaps introduced to maximize homology; *, bases constituting the -35 and -10 boxes; √, bases constituting the anticodon; ∇, position of the *Tn10* insertion on the *S. enteritidis* 3b-122 chromosome. The DNA sequence corresponding to the proposed mature tRNA^{Arg} (UCU) is underlined. (B) Diagram of the proposed secondary structure for tRNA^{Arg} (UCU) from *S. enteritidis* 3b. The anticodon bases are underlined.

quences listed in the NCBI databases showed that the sequence was identical to that of *fimU* of *S. typhimurium* (Fig. 1C and 2B) and 96% similar to that of *argU/dnaY* of *E. coli* (Fig. 2A). These genes encode arginine-specific tRNAs that recognize the rare AGA codon. The nucleotide sequence of *fimU* from *S. enteritidis* 3b contained 4 inverted repeats, which were predicted to fold the sequence into the characteristic tRNA-like cloverleaf structure (Fig. 2B) with UCU in the expected tRNA anticodon position. Together, these data suggested that *fimU* from *S. enteritidis* 3b encoded an arginine-specific tRNA.

Analysis of the nucleotide sequence downstream of *fimU* revealed an open reading frame oriented in the opposite direction such that the 3' ends of the two genes overlapped. The predicted amino acid sequence was 88% similar to that of the prophage DLP12 integrase of *E. coli*. The sequence further downstream of *fimU* displayed 60 to 88% similarities to those encoding transposases of the IS3 family of insertion elements.

Mapping *fimU* on the *S. enteritidis* 3b genome. Like *fimA*, *fimU* was localized to chromosomal *XbaI* and *BlnI* fragments in the 98.5- to 13.0-C's region of the chromosome in both *Salmonella* serovars. By using a series of *S. typhimurium* and *S. enteritidis* *Tn10* mutants the *fimU* gene was more precisely mapped to between *purE884::Tn10* at 12.6 C's and the first *XbaI* restriction site at 13.6 C's in *S. enteritidis* or 13.0 C's in *S. typhimurium*. Thus, *fimU* mapped to the same region shown previously to contain the *fimA* gene in the *fim* gene cluster.

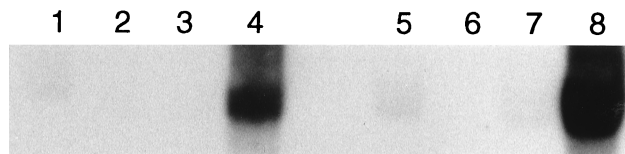


FIG. 3. Northern blot analysis of tRNA^{Arg} (UCU) production in *S. enteritidis* 3b strains. A *fimU*-specific probe was hybridized to PAGE-separated total RNA from *S. enteritidis* 3b (lanes 1 and 5), 3b-122 (lanes 2 and 6), 3b-122 pGEM-T1 (lanes 3 and 7), or 3b-122 pLU/TA 4-1 (lanes 4 and 8). Lanes 1 to 4 contain 25 µg of RNA, and lanes 5 to 8 contain 50 µg of RNA.

Analysis of *fimU* transcription. To determine if the *fimU* transcript was the same size as tRNA, a *fimU*-specific probe was hybridized to a blot containing total RNA isolated from *S. enteritidis* 3b, 3b-122, 3b-122 pGEM-T1, or 3b-122 pLU/TA 4-1 grown under conditions optimal for type 1 fimbria (SEF21) production in *S. enteritidis* 3b (static LB broth, 48 h, 37°C). The *fimU*-specific probe hybridized to a transcript that was present in total RNA from 3b and 3b-122 pLU/TA 4-1 (Fig. 3). The *fimU* transcript was consistently difficult to detect on Northern blots of 3b RNA even with excessive amounts of RNA loaded on the gels (25 µg [Fig. 3, lanes 1 to 4] and 50 µg [Fig. 3, lanes 5 to 8]) and extended exposure of the blots to X-ray film. Although the transcript was not found in RNA prepared from 3b-122, trace levels were evident in 3b-122 carrying the vector pGEM-T (Fig. 3), but the transcript was even more difficult to detect than its counterpart in 3b. The transcript detected with the *fimU*-specific probe comigrated with tRNA, suggesting that the product of *fimU* from *S. enteritidis* was indeed a tRNA molecule.

Complementation of fimbrin expression and fimbria assembly in 3b-122. Fimbria expression was examined in *S. enteritidis* 3b, 3b-122, 3b-122 pGEM-T1, and 3b-122 LU/TA 4-1 grown under conditions that promoted production of both SEF14 and SEF21 by the wild-type strain (static CFA broth, 48 h, 37°C). Western blot analysis of whole-cell lysates using SEF14- or SEF21-specific antisera showed that complementation of the insertion mutation in 3b-122 with pLU/TA 4-1 restored SEF14 and SEF21 fimbria expression (Fig. 4). Thus *fimU* affected the production of two fimbrins encoded by genes located on two different gene clusters.

Assembly of SEF14 and SEF21 fimbriae on the cell surface of *S. enteritidis* 3b, 3b-122, 3b-122 pGEM-T1, or 3b-122 pLU/TA 4-1 was determined by immunogold labelling and electron microscopy performed on cells grown in static CFA broth for 48 h at 37°C. The wild-type strain, *S. enteritidis* 3b, expressed both SefA and FimA and assembled the respective subunits into SEF14 and SEF21 fimbriae (Table 2). Similar analyses of 3b-122 and 3b-122 pGEM-T1 showed that SEF14

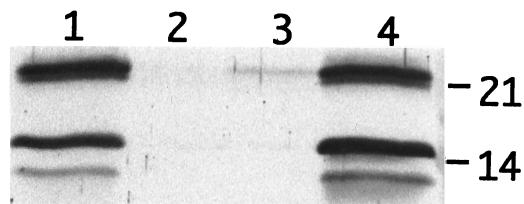


FIG. 4. Complementation analysis of *S. enteritidis* 3b-122 Tn10 mutant with the *fimU*-containing recombinant plasmid pLU/TA 4-1. Whole-cell extracts were analyzed by Western blotting to determine the presence of SefA (21 kDa) and FimA (14 kDa) fimbrin proteins in *S. enteritidis* 3b (lane 1), 3b-122 (lane 2), 3b-122 pGEM-T1 (lane 3), and 3b-122 pLU/TA 4-1 (lane 4). Numbers at right indicate positions of SefA (21) and FimA (14).

TABLE 2. Detection of assembled SEF14 and SEF21 fimbriae in various *S. enteritidis* strains by immunoelectron microscopy with specific antifimbrial sera

<i>S. enteritidis</i> 3b strain	Fimbria production ^a		
	SEF14	SEF21	SEF17
Wild type	++	+	+++
3b-122	—	± ^b	+++
3b-122 pGEM-T1	—	± ^c	+++
3b-122 pLU/TA 4-1	+++	+	+++

^a The percentages of cells labelled by immunogold are indicated as follows: —, none; ±, less than 10%; +, 10 to 50%; ++, 50 to 75%; +++, 75 to 100%.

^b Two of approximately 400 cells were densely labeled.

^c Two of approximately 600 cells were sparsely labeled.

was not produced and that SEF21 fimbriae on the cell surfaces of these two strains were rarely detected (Table 2), a result consistent with the Western blot data (Fig. 4). In contrast, SEF14 and SEF21 fimbriae were evident on the surface of 3b-122 pLU/TA 4-1 (Fig. 5) at levels equal to or greater than that on 3b. Thus, expression of SefA and FimA fimbrins and assembly of their respective fimbriae were restored by complementation of the Tn10 *fimU* mutation in 3b-122 with a wild-type copy of the *fimU* gene on pLU/TA 4-1. Cells producing SEF17 were readily seen without immunolabelling on all the grids prepared for electron microscopy (Table 2).

Analysis of *sefA* transcription. The effect of tRNA^{Arg} (UCU) on *sefA* transcript production was analyzed by hybridizing a *sefA*-specific probe to a blot containing total cellular RNA isolated from *S. enteritidis* 3b, 3b-122, 3b-122 pGEM-T1, or 3b-122 LU/TA 4-1 grown under conditions optimal for SEF14 production in 3b (static CFA broth, 48 h, 37°C). The *sefA*-specific probe hybridized to a 660-base transcript that was present in RNA from 3b and 3b-122 pLU/TA 4-1 but absent from RNA from 3b-122 and 3b-122 pGEM-T1 (Fig. 6). The strains expressing the *sefA* transcript corresponded to those carrying a functional *fimU* gene, suggesting that *fimU* was required for expression of *sefA*.

DISCUSSION

fimU, located in the *fim* (*sef21*) operon of *S. enteritidis* 3b, encodes an arginine-specific tRNA that is required for expression of not only SEF21 fimbriae (type 1) but also SEF14 fimbriae. The product of *fimU* in 3b is a tRNA, since Northern blot analysis of RNA from 3b and 3b-122 pLU/TA 4-1 demonstrates that the *fimU* transcript comigrates on polyacrylamide gels with tRNA. Furthermore, the 77-nucleotide sequence of the *fimU* gene of 3b is identical to that of *fimU* of *S. typhimurium* (39) and shares extensive homology with that of *argU* encoding tRNA^{Arg} (UCU) from *E. coli* (18). The *fimU*-encoded transcript from 3b can be folded into a typical tRNA cloverleaf structure containing the 3'-terminal sequence CCA as well as the invariant or semivariant nucleotides common to tRNA molecules (19, 34). Finally, the DNA sequence 5' to the *fimU* gene contains features common to the promoters of tRNA operons including the consensus *E. coli* -10 and -35 promoter elements (16, 21) and a G+C-rich discriminator sequence (16, 42, 43). The regulatory mechanisms controlling *fimU* expression are unknown. Recently, however, the integration and excision of plasmids, phage, and pathogenicity islands into and out of the chromosomes at tRNA loci have been shown to affect tRNA gene expression (20, 33). As shown with the *E. coli* tRNA gene *argU* (25), the open reading frame adjacent to and overlapping *fimU* is a homolog of the integrase

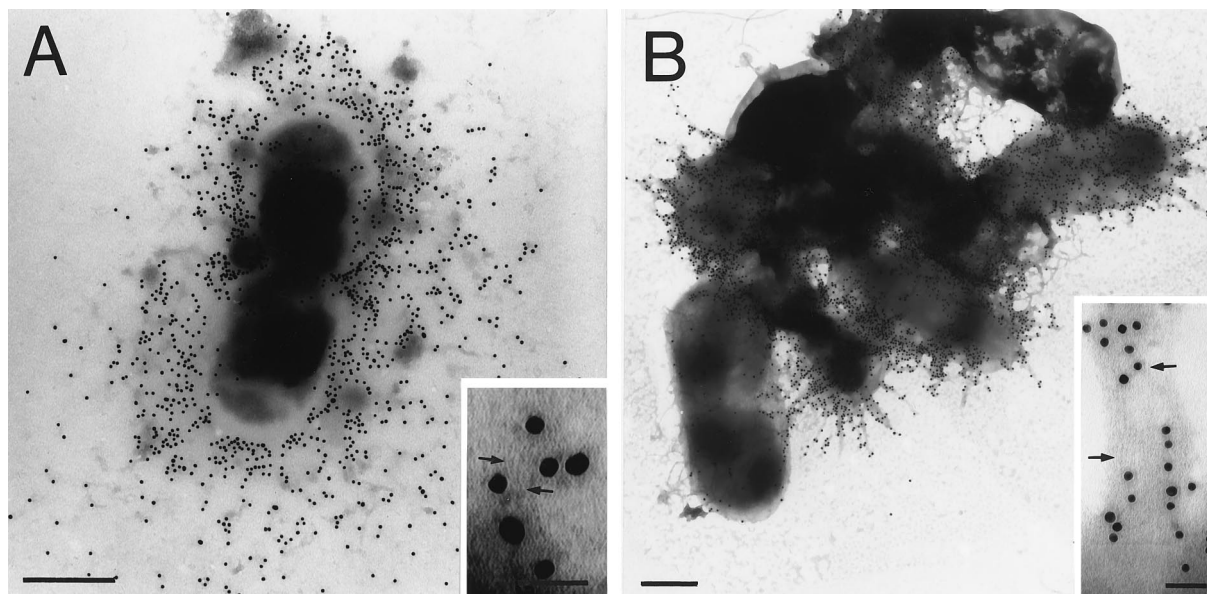


FIG. 5. Analysis of SEF14 and SEF21 fimbria assembly in *S. enteritidis* 3b-122 pLU/TA 4-1 by immunogold electron microscopy. *S. enteritidis* 3b-122 pLU/TA 4-1 was labeled with protein A-gold and negatively stained following incubation with immune serum generated to SEF14 (A) or SEF21 (B). Arrows indicate individual immunogold-labeled SEF14 and SEF21 fimbriae in panel A and B insets, respectively. The average diameter of the gold particles was 15 nm. Bar, 0.5 μ m (electron micrograph) or 50 nm (inset).

gene (*int*) from the defective lambdoid prophage DLP12. Integration of prophage DLP12 at this site prevents cotranscription of the *int* gene with *fimU*, which may contribute to the regulation of *fimU* expression in *S. enteritidis* 3b.

The influence of tRNA^{Arg} (UCU) encoded by *fimU* on SEF14 and SEF21 fimbria production is evident in the Tn10 insertion mutant *S. enteritidis* 3b-122. The transposon, inserted between the predicted promoter and the 5' end of the mature *fimU* transcript, disrupts transcription of *fimU* and thus tRNA^{Arg} (UCU) production. This mutation results in the loss of SefA production and selective loss of FimA production, i.e., the subunits of SEF14 and SEF21 (type 1) fimbriae, respectively. Thus, in *S. enteritidis* 3b, tRNA^{Arg} (UCU) is required for SEF14 production and enhances type 1 fimbria (SEF21) production. In *E. coli*, cross-talk has also been reported to occur between adhesin gene clusters (29), and tRNA molecules have been shown to play a key role in global regulatory cascades (20). However, this is the first study to show that a tRNA-specific locus found on one fimbrial operon influences the production of two fimbrins whose operons are separated by 15 C's on the chromosome.

tRNA^{Arg} (UCU) encoded by *fimU* is required for transcription of *sefA*, the gene encoding the subunit of SEF14 fimbriae in *S. enteritidis* 3b. The regulatory mechanism is unknown, but a direct correlation between the abundance of tRNAs and the

occurrence of the respective codons in protein genes (22, 23) has been suggested to control the translation of genes containing rare codons (3, 37). Since AGA, the codon recognized by the tRNA^{Arg} (UCU) species encoded by *fimU*, is one of the least-used codons for arginine, then perhaps the limited availability of charged tRNAs for this minor codon controls the level of translation of the *sefA* transcript or of a transcript whose protein product is involved in the regulation of *sefA* transcription. *sefA* contains neither of the rare arginine codons AGA or AGG recognized by tRNA^{Arg} (UCU), indicating that *fimU* expression would not have a direct effect on the translation of *sefA* mRNA. However, *sefE*, the gene encoding the putative AraC-like transcriptional activator of the *sef14* gene cluster, contains 13 arginine codons, including 9 AGA codons and 1 AGG codon (5). Perhaps the tRNA^{Arg} (UCU) encoded by *fimU* regulates translation of *sefE*, which would in turn affect transcription of *sefA* and the downstream genes.

With the exception of the gene *fimA* encoding the subunit of SEF21 fimbriae (12), the remainder of the *sef21* gene cluster has not been characterized in *S. enteritidis* 3b. Thus, the mechanism for regulation of type 1 fimbria synthesis by *fimU* remains to be determined. However, type 1 fimbria (SEF21) production is optimal when *S. enteritidis* 3b is grown at 37°C in a static broth culture but suboptimal when the cells are grown at lower temperatures (21 to 30°C) in shaking broth culture or on solid medium (12). Similarly, expression of SEF14 fimbriae by *S. enteritidis* 3b is environmentally controlled by temperature, medium composition, and aeration, and is optimal at 37°C in static, aerobic CFA broth (5). Thus, the coregulation of SefA and FimA fimbrin production by *fimU*-encoded tRNA^{Arg} (UCU) results in the corresponding fimbriae being expressed under similar environmental conditions, which may give the bacteria a competitive advantage for survival.

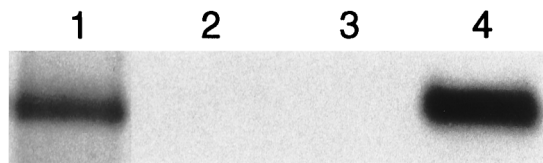


FIG. 6. Northern blot analysis of *sefA* transcription in *S. enteritidis* 3b strains. A *sefA*-specific probe was hybridized to 10 μ g of total RNA from *S. enteritidis* 3b (lane 1), 3b-122 (lane 2), 3b-122 pGEM-T1 (lane 3), or 3b-122 LU/TA 4-1 (lane 4).

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