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Insertional Inactivation of an *Escherichia coli* Urease Gene by IS*3411*

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Ureolytic *Escherichia coli* are unusual clinical isolates that are found at various extraintestinal sites of infection, predominantly the urinary tract. The urease-positive phenotype is unstable in approximately 25% of these isolates, and urease-negative segregants are produced at a high frequency. We have studied the nature of the urease-positive-to-negative transition in one of these isolates, designated *E. coli* 1021. Southern hybridization experiments with genomic DNA extracted from seven independent *E. coli* 1021 urease-negative segregants revealed the presence of a 1.3-kb DNA insertion in the urease gene cluster. A DNA fragment containing the DNA insertion was cloned from one of the urease-negative segregants. This cloned DNA fragment was capable of mediating cointegrate formation with the conjugative plasmid pOX38, suggesting that the DNA insertion was a transposable element. The insert was identified as an IS*3411* element in *ureG* by DNA sequence analysis. A 3-bp target duplication (CTG) flanking the insertion element was found. DNA spanning the insertion site was amplified from the other six urease-negative segregants by using the polymerase chain reaction. The DNA sequence of the amplified fragments indicated that an IS*3411* element was found in an identical site in all urease-negative segregants examined. These data suggest that in *E. coli* 1021, IS*3411* transposes at a high frequency into *ureG* at a CTG site, disrupting this gene and eliminating urease activity.

Many species of plants, fungi, and bacteria produce urease, which hydrolyzes urea to form carbonic acid and ammonia and thereby provides an easily assimilated nitrogen source for the organism (14, 28). Urease-producing bacteria are found in various environments, including soil, water, rumens, and mammalian intestinal tracts. The prevalence of urease-producing species is not surprising, given the ubiquitous presence of urea. Urea is the principal nitrogenous waste product of mammals, and therefore is found in abundance in both soil and aqueous environments.

In recent years much has been learned about bacterial ureases and the genes encoding these enzymes. Urease genes have been isolated from *Escherichia coli* (7), *Proteus mirabilis* (20, 41), *Proteus vulgaris* (30), *Morganella morganni* (15), *Klebsiella pneumoniae* (12), *Klebsiella aerogenes* (31), *Providencia stuartii* (29, 32), *Staphylococcus saprophyticus* (11), *Ureaplasma urealyticum* (2, 3), and *Helicobacter pylori* (5, 24). In the members of the family *Enterobacteriaceae*, the genetic organization of the urease genes appears to be conserved in all species examined thus far. Nucleotide sequence analysis has identified six genes in the *K. aerogenes* (31) and *P. mirabilis* (21) urease gene clusters. Each cluster contains a *ureA*, *ureB*, and *ureC* gene; these genes encode the urease structural polypeptides termed γ (11 kDa), β (12 kDa), and α (60 to 61 kDa), respectively. One copy of the α polypeptide combines with two copies each of γ and β polypeptides to form a urease subunit. In *P. mirabilis* and *K. aerogenes*, two subunits come together to constitute a mature urease enzyme of approximately 220 kDa (28).

Urease is a cytoplasmic enzyme (with the exception of *H. pylori* urease, which is associated with the cell surface [8]). A recombinant plasmid encoding only *K. aerogenes ureA*, *ureB*, and *ureC* results in the production of cytoplasmic urease subunits but not an active enzyme (31). If three additional genes, *ureE*, *ureF*, and *ureG*, are present in *trans*, then a functional enzyme is produced. The exact role of these accessory genes is not known at this time. Urease is a nickel metalloenzyme, and *K. aerogenes* urease contains two nickel ions per subunit (38, 39). It is thought that one function of the accessory polypeptides is to facilitate the association of nickel with the urease subunits. The *P. mirabilis* urease gene cluster contains two genes found upstream of *ureA*, *ureD*, and *ureR* that are believed to encode regulatory proteins (21, 33, 35).

Urease contributes to the virulence of bacterial uropathogens when expressed in the urinary tract (4, 10, 13, 19, 34). It was this association with urinary tract virulence that first caused us to examine bacterial urease genes. We have been studying the urease genes of uropathogenic *E. coli* and have previously shown that there are two distinct urease loci found in these *E. coli* (7). One locus is plasmid encoded and, in addition to being found in *E. coli*, can be found in urease-producing *Salmonella* and *Providencia* species. The other locus is found in approximately half of the urease-producing *E. coli* tested and appears to be chromosomally encoded.

Approximately 25% of ureolytic *E. coli* give rise to urease-negative variants at a high frequency (40). We have studied the nature of this urease-positive-to-negative transition in *E. coli* 1021, which contains a chromosomally encoded urease locus. We have previously reported that urease-positive-to-negative transition in *E. coli* 1021 is associated with a DNA rearrangement (6). Here we show that this rearrangement reflects the site-specific insertion of IS*3411* into *ureG*, a gene encoding one of the nonstructural proteins associated with the urease gene cluster.

MATERIALS AND METHODS

Bacterial strains and media. *E. coli* DH5α [F$^-$ *endAI hsdR17* (r_K^- m_K^-) *supE44 thi-1* λ^- *recAI gyrA96 relAI* ϕ80 Δ*lacZ*ΔM15] was the host for all recombinant plasmids used

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in this study. *E. coli* HB101 (F$^-$ *hsdR hsdM recA13 lacY1 leuB6 ara-14 galK2 proA2 thi-1* Sm^r Rif^r) was used as the bacterial recipient in experiments measuring cointegrate formation. Clinical isolates were either from the State of California Department of Health, Berkeley, Calif., or from Jackson Memorial Hospital, Miami, Fla.

Urease-positive and urease-negative isolates were distinguished by using Christensen urea agar slants, urea segregation agar, or urea-glucose-eosin Y-methylene blue agar (UGEM) as previously described (6, 7). Bacteria were grown in L broth or on L broth solidified with 1.5% agar. Media were supplemented with carbenicillin (100 μg/ml) when necessary.

Isolation of *E. coli* 1021 urease-negative segregants. Individual urease-positive *E. coli* 1021 colonies were patched onto L agar plates and allowed to grow overnight at 37°C. The plates were stored at 4°C, and at various times an inoculum from each of the individual colonies was streaked onto UGEM agar plates to screen for urease-negative cells. After 1 week, 15% of the original colonies contained urease-negative segregants.

Nucleic acid isolation and construction of recombinant plasmids. Plasmid DNA was isolated by alkaline lysis. Whole-cell DNA was isolated as previously described (27). Restriction enzymes were purchased from either New England BioLabs, Inc., Beverly, Mass., or Bethesda Research Laboratories, Inc., Gaithersburg, Md. Enzymatic reaction conditions were those recommended by the manufacturer.

Plasmid vectors were digested with the appropriate restriction enzymes and ligated to DNA fragments isolated from low-melting-point agarose by using T4 DNA ligase (Bethesda Research Laboratories). Recombinant plasmids were transformed into competent *E. coli* DH5α, and the resulting transformants were screened by colony hybridization (26).

DNA sequence analysis. DNA was sequenced by the dideoxy method of Sanger et al. (36) as modified for use with Sequenase (U.S. Biochemicals). DNA fragments to be sequenced were cloned into either pSP72 or pGEM-7Zf(−) (Promega Corporation, Madison, Wis.). Double-stranded DNA templates were sequenced by using oligonucleotide primers homologous to either the T7 or the SP6 promoters encoded on the plasmids or with primers designed internal to the cloned DNA fragment. [^{35}S]dATP-labelled DNA fragments were separated by electrophoresis on 8% acrylamide gels and visualized by autoradiography.

Southern hybridization. Whole-cell DNA digested with various restriction enzymes was fractionated by agarose gel electrophoresis, transferred to nitrocellulose sheets (Schleicher and Schuell, Inc., Keene, N.H.) by the Southern technique (37) and hybridized under stringent conditions as described previously. After hybridization, the nitrocellulose sheets were washed in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) with 0.1% sodium dodecyl sulfate at 67°C. Probes were labelled with [^{32}P]dCTP (Dupont, NEN Research Products, Boston, Mass.) by nick translation. Hybridization was revealed by autoradiography with XAR-Omat films (Eastman Kodak Co., Rochester, N.Y.) in the presence of an intensifying screen. DNA fragments used as probes were isolated from low-melting-point agarose gels.

When necessary after autoradiography, blots were washed in 2.5 mM NaOH for 6 h at room temperature to remove the radioactive probe. Blots were than rinsed in 2× SSC and hybridized as described above.

Cointegrate formation. Cointegrate formation was monitored by measuring the cotransfer of the conjugative F plasmid derivative pOX38 (Cm^r) (23) and pUM101 (Amp^r), containing IS*3411*, from a donor to a recipient *E. coli* strain. The cotransfer of pUM102 (Amp^r), which does not contain IS*3411*, and pOX38 was monitored as a negative control. *E. coli* DH5α pOX38 containing either pUM101 or pUM102 was used as the donor in a mating experiment with a rifampin-resistant strain of *E. coli* HB101 as the recipient. Matings were performed at 37°C in broth for 4 h and plated on L agar supplemented with carbenicillin (100 μg/ml), chloramphenicol (20 μg/ml), or both antibiotics. The frequency of cointegrate formation was expressed as the frequency of cotransfer of Amp^r (pUM101 or pUM102) and Cm^r (pOX38) compared with the frequency of Cm^r (pOX38) transfer alone.

DNA amplification with the polymerase chain reaction. DNA spanning the IS*3411* insertion site in *ureG* was amplified for DNA sequence analysis with the polymerase chain reaction (9). Oligonucleotide primers corresponding to a region in *ureG* (5′-TTGATCACGAGGAAGTCGGA-3′) and a region in IS*3411* (5′-CGTGGGTGGACTGGTATAA-3′) were incubated with whole-cell DNA (0.2 μg) and the fragment internal to the two primers was amplified with *Taq* DNA polymerase (Perkin Elmer Cetus, Norwalk, Conn.). Reaction conditions were as described by the manufacturer with the exception that the annealing temperature used was 50°C. DNA was amplified for 25 cycles. Amplified DNA was purified from the reaction components by centrifugation through a Centricon 30 column (Amicon, Danvers, Mass.). DNA fragments were then sequenced directly by using a third oligonucleotide (5′-CTTTCCACAAAGATCAG-3′), corresponding to a region in *ureG*.

RESULTS

***E. coli* 1021 urease gene cluster.** *E. coli* 1021 is a urease-producing isolate obtained from a patient with a urinary tract infection. The *E. coli* 1021 urease gene cluster was isolated as a 9.4-kb *Hin*dIII fragment insert in pBR322 and the recombinant plasmid was termed pURE. Unidirectional deletions originating at either end of the 9.4-kb *Hin*dIII fragment indicate that 4.6 kb of this fragment is necessary for the expression of urease (Fig. 1A) (7). The DNA sequence of 2.5 kb of this 4.6-kb region was determined (data not shown). The sequences showed between 75 and 87% nucleotide residue identity with the corresponding *K. aerogenes* urease gene sequences (31). On the basis of this homology, we have assigned the positions of the strain 1021 urease genes as shown in Fig. 1A. The positions of these genes correspond to the positions of the genes determined previously by in vitro transcription-translation experiments with pURE and various pURE derivatives containing transposon-insertion mutations (6).

Urease-positive-to-negative transition is associated with a 1.3-kb insertion. The urease-positive phenotype in *E. coli* 1021 is unstable, and urease-negative segregants are produced at a high frequency. Previously, we have shown that the urease-positive-to-negative transition in *E. coli* 1021 is associated with a DNA rearrangement (6).

As an initial step to determine the nature of this rearrangement, seven urease-negative segregants were isolated (see Material and Methods). Genomic DNA from one of the *E. coli* 1021 urease-negative segregants (*E. coli* 1021-G) was compared by Southern hybridization with genomic DNA of wild-type *E. coli* 1021. The DNA was digested with combinations of six different enzymes known to have restriction sites in or near the urease gene cluster. Various pURE-

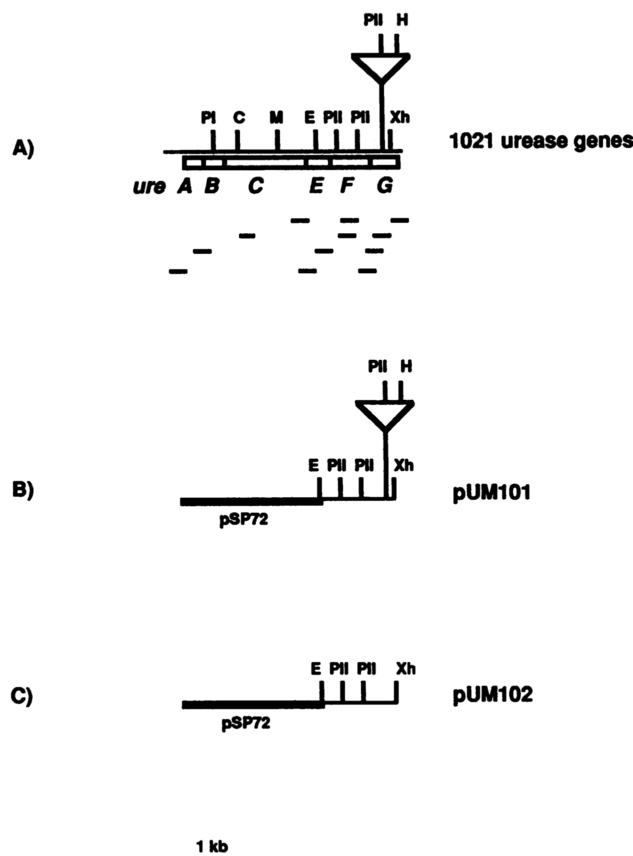

FIG. 1. *E. coli* 1021 urease genes. (A) Physical and genetic map of the *E. coli* 1021 urease locus. Open boxes indicate positions of the six urease genes, designated *ureA*, *-B*, *-C*, *-E*, *-F*, and *-G*, as determined by DNA sequence analysis. Sequencing was as described in Materials and Methods. Short horizontal lines represent fragments of DNA sequenced. (B) Map of pUM101. (C) Map of pUM102. C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; M, *Mlu*I; PI, *Pvu*I; PII, *Pvu*II; X, *Xho*I.

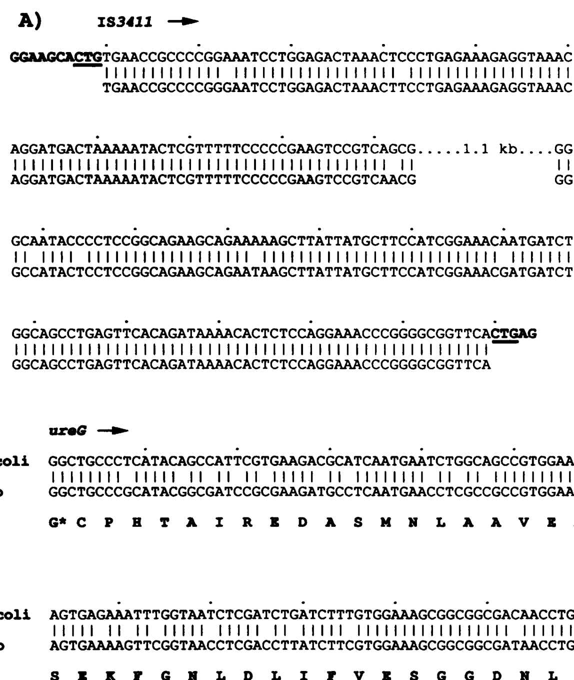

FIG. 2. Partial DNA sequence of IS*3411* and *ureG*. (A) Top line is the left and right end sequence of IS*3411* element found in *ureG*. Bold type is *ureG* sequence. The CTG target site is underlined. Bottom line corresponds to the sequence of the left and right ends of IS*3411* as determined by Ishiguro and Sato (17). (B) DNA sequence of *ureG* containing IS*3411* target site. Sequence is compared with the *K. aerogenes ureG* sequence. Consensus amino acid sequence from region is shown in boldface type. *, correspondence to amino acid 71 of *ureG* protein product.

derived DNA fragments were used as hybridization probes. Comparison of the hybridization patterns obtained with the wild-type DNA and the *E. coli* 1021-G DNA indicated that the rearrangement was a 1.3-kb DNA insertion into a region just to the left of the *Xho*I site in the urease locus (Fig. 1A). Hybridization analysis indicated that the 1.3-kb DNA insert contained a *Hin*dIII and a *Pvu*II restriction site.

Since it was determined that the DNA insertion resided on a 2.8-kb DNA fragment flanked by *Xho*I and *Eco*RI restriction sites, the region containing the insertion was isolated from *E. coli* 1021-G in the following manner. *E. coli* 1021-G DNA was digested to completion with *Xho*I and *Eco*RI and size fractionated through agarose. The 2.8-kb fragments were isolated, and a bank of these fragments was produced by using the recombinant cloning vector pSP72 (Promega Corp.). The resultant *E. coli* DH5α transformants were screened by colony hybridization with the *Xho*I-to-*Eco*RI fragment from pURE as the probe. A resultant recombinant plasmid containing the DNA insertion within the *Xho*I-to-*Eco*RI fragment from the urease locus was isolated and termed pUM101 (Fig. 1B). Coincident with this construction, the 1.5-kb *Xho*I-to-*Eco*RI DNA fragment from the *E. coli* 1021 urease-positive locus was cloned into pSP72, and the resultant plasmid was termed pUM102 (Fig. 2B).

The DNA sequence of pUM101 (excluding the vector) was determined and compared with the DNA sequence of pUM102. This analysis indicated that the DNA insertion on pUM101 resided 150 bp upstream of the *Xho*I site. We sequenced 94 bp of the left end of the inserted DNA and 114 bp of the right end. The GenBank (1) data base was scanned for sequences similar to the insert sequences; these sequences showed 98 and 97% nucleotide residue identity to the left and right ends, respectively, of the IS*3411* DNA sequence determined by Ishiguro and Sato (Fig. 2A) (GenBank accession no. M19532) (17). The IS*3411* sequence reported by Ishiguro and Sato contains a *Pvu*II site at bp 770 and a *Hin*dIII site at bp 1226. The presence and positions of these sites correspond with the positions of the *Hin*dIII and *Pvu*II sites found within the DNA insertion on pUM101. The sequence analysis indicated that the right end of IS*3411* as defined by Ishiguro and Sato resides closest to the *Xho*I site.

IS*3411* transposes into *ureG* at a CTG target site. Comparison of the sequences for pUM101 and pUM102 indicated that a CTG nucleotide triplet was duplicated upon insertion of IS*3411*. This suggests that CTG was the target recognition site. The DNA sequence of the region flanking IS*3411* was compared with the DNA sequence of the *K. aerogenes* urease gene cluster (31). The 130 bp surrounding the insertion site showed 83% nucleotide residue identity to the DNA sequence of *K. aerogenes ureG* (Fig. 2B). When translated, this region shows 100% amino acid residue identity to amino acids 71 to 114 of the *K. aerogenes ureG* gene product.

Ishiguro and Sato showed that IS*3411* was capable of mediating cointegrate formation between two replicons (16,

DNA source	Sequence
pUM101	GGGGCGGTTCACTGAGTGA
1021-G	GGGGCGGTTCACTGAGTGA
1021-B	GGGGCGGTTCACTGAGTGA
1021-3	GGGGCGGTTCACTGAGTGA
1021-12	GGGGCGGTTCACTGAGTGA
1021-13	GGGGCGGTTCACTGAGTGA
1021-14	GGGGCGGTTCACTGAGTGA
1021-15	GGTTCACTGAGTXA

FIG. 3. DNA sequence of polymerase chain reaction-generated fragments. Shown is the sequence of DNA fragments containing the IS*3411* insertion site generated from pUM101 and seven independently isolated *E. coli* 1021 urease-negative segregants (1021-G, -B, -3, and -12 to -15). Underlined sequence corresponds to the right end of IS*3411*; plain text corresponds to *ureG*. Shown is the inverse complement of the actual sequence obtained.

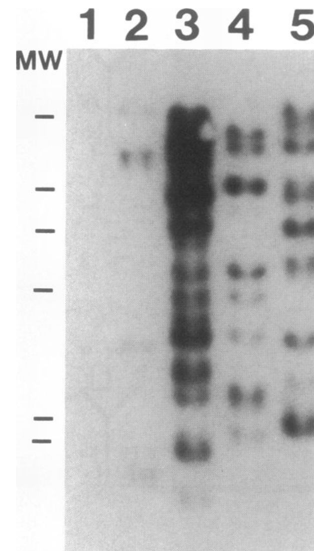

FIG. 4. IS*3411* hybridization profile of various urease-positive *E. coli* strains and *E. coli* DH5α. Genomic DNA (4 μg) from designated strains was digested with *Hin*dIII and hybridized under stringent conditions to an IS*3411* DNA probe. After initial hybridization, the blot was stripped of the original probe and rehybridized to pBR322 to control for contaminating *bla* sequences in the IS*3411* probe preparation. Lanes: 1, *E. coli* DH5α; 2, *E. coli* 1021; 3, *E. coli* 5406; 4, *E. coli* 914; 5, *E. coli* 187; MW, molecular weight markers.

17). The generation of cointegrates is believed to be evidence of replicative transposition. The IS*3411* element on pUM101 was tested for transposition capabilities by measuring cointegrate formation between pUM101 and pOX38 (a derivative of the conjugative plasmid F deleted of all insertion elements [23]) as described in Materials and Methods. pUM101 was capable of forming a cointegrate with pOX38; cotransfer of pUM101 and pOX38 from a *recA* mutant donor strain into a recipient strain occurred at a frequency of 10^{-4} cells per generation. The cotransfer of pOX38 and pUM102, occurred at a frequency of $<10^{-6}$ cells per generation. Therefore, we conclude that the IS*3411* element on pUM101 was capable of transposition and that the inactivation of urease in *E. coli* 1021-G was due to the transposition of IS*3411* into *E. coli ureG*.

Insertion of IS*3411* into *ureG* is site specific. Southern hybridization analysis was performed by using genomic DNA from the six other independently isolated *E. coli* 1021 urease-negative segregants (data not shown). The results of these analyses indicated that there was an insertion similar to that found in *E. coli* 1021-G in all six strains. Each strain contained a 1.3-kb insertion just to the right of the *Xho*I site.

The exact site of insertion was determined for the six independently isolated urease-negative segregants. By using the polymerase chain reaction, DNA spanning the insertion site was amplified from the urease-negative segregants, with pUM101 and *E. coli* 1021-G DNA as controls. One of the oligonucleotide primers corresponded to a region in *ureG*, and the other corresponded to a site in IS*3411*. The resultant DNA fragments were sequenced directly by using a third oligonucleotide corresponding to a site in *ureG* as the sequencing reaction primer. The insertion of IS*3411* into *ureG* occurred in an identical site in all seven isolates examined (Fig. 3).

***E. coli* isolates containing chromosomal urease gene clusters contain multiple copies of IS*3411*.** *E. coli* 1021 contains at least five copies of IS*3411*, as determined by hybridization analysis with an IS*3411* DNA probe (Fig. 4). Three other clinical *E. coli* isolates (*E. coli* 5406, 914, and 187), each containing urease gene clusters homologous to the 1021 gene cluster, also contained multiple copies of IS*3411* (Fig. 4). The biochemical characteristics and hybridization profiles of these isolates were nonidentical, suggesting that they are distinct strains. Fifteen urease-negative clinical *E. coli* isolates (nine from urinary tract infections), two urease-producing *Salmonella* spp., two urease-producing *Providencia* strains, and the urease-negative *E. coli* K-12 strains DH5α and W3100 were tested for the presence of IS*3411*. Four of the clinical *E. coli* strains showed either one or two bands of homology. The *E. coli* K-12 strains and the 15 other clinical isolates (including *E. coli*, *Salmonella* spp., and *Providencia* spp.) showed no homology. This suggests that IS*3411* elements may be found more often in *E. coli* isolates that contain the chromosomal urease gene cluster.

Because of the presence of multiple copies of IS*3411*, *E. coli* 5406, 914, and 187 were screened for the stability of the urease phenotype. Urease-negative segregants were not produced at a detectable frequency in these isolates. We checked for the presence of the CTG target site in *ureG* of these isolates. DNA corresponding to the target site was amplified as described above, and the DNA sequence of the amplified fragment was determined. There was no difference between the sequences of the amplified fragments from *E. coli* 914, 187, and 1021. (The DNA sequence of *E. coli* 5406 was not determined.)

Map of the region surrounding the *E. coli* 1021 urease genes. Urease is an unusual trait in *E. coli*. This fact in conjunction with the association between IS*3411* and ureolytic *E. coli* 1021 suggested to us that the urease trait might be encoded on a composite transposon flanked by IS*3411* elements. We screened the various original cosmid clones encoding the *E. coli* 1021 urease genes for the presence of IS*3411* homologous sequences. We found evidence of an IS*3411* element residing approximately 5.5 kb upstream from the gene cluster. However, we were unable to find a flanking element (Fig. 5). Therefore, we cannot conclude at this time

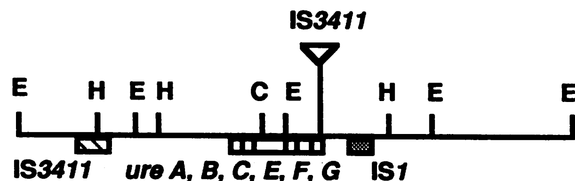

FIG. 5. Map of *E. coli* 1021 urease genes and surrounding region. Positions of insertion sequences IS*3411* and IS*1* are as shown. C, *Cla*I; E, *Eco*RI; H, *Hin*dIII.

that the association between the urease genes and IS*3411* is due to a urease encoding transposon.

In a previous publication, we had shown that there was a DNA sequence that resides downstream of *ureG* that is repeated on the *E. coli* chromosome. This sequence was determined by hybridization analysis to be IS*1*. In addition, the restriction endonuclease cleavage sites of this repetitive sequence correspond to the sites found on IS*1*. The location of IS*1* relative to the urease genes is as shown in Fig. 5. It does not appear that this IS*1* is involved in the urease-positive-to-negative transition seen in *E. coli* 1021.

DISCUSSION

In this report we present evidence that the urease-positive-to-negative transition in *E. coli* 1021 is due to the site-specific transposition of IS*3411* into *ureG*. Inactivation of bacterial genes by IS elements is not unusual; in fact, IS elements were first recognized by their ability to produce mutations in the *gal* and *lac* operons of *E. coli* (22, 25). This is the first reported case of which we are aware, however, of insertional inactivation of a gene by IS*3411*. IS*3411* has been shown to constitute the ends of the composite transposon Tn*3411*, which encodes citrate utilization genes (16–18). *E. coli* 1021 and the other three urease-positive *E. coli* isolates examined here all contained multiple copies of IS*3411* but were citrate utilization negative, suggesting that the IS*3411* elements found in these isolates were not associated with Tn*3411*.

The complete nucleotide sequence of IS*3411* was published by Ishiguro and Sato (17). The portion of IS*3411* we sequenced is 97 to 98% identical to the published sequence. We show that the left and right ends of IS*3411* contained a 27-bp repeat, with a 4-bp mismatch between the repeated ends, while the element studied by Ishiguro and Sato has a 3-bp mismatch between the repeated ends. The target site found here for IS*3411* insertion was CTG, which differs from the GAA target site for IS*3411* reported previously. Although they differ in composition, both target sequences are 3 bp in length.

It is interesting to us that both *E. coli* 914 and 187 contain multiple copies of IS*3411* and the CTG target site, yet there was not a high frequency of transposition of IS*3411* into this site in these isolates. The IS*3411* sequences in *E. coli* 914 and 187 were detected by Southern hybridization, and therefore it is not clear if they are capable of transposition. However, it is unlikely that all the IS*3411* sequences found in these strains are nonfunctional. Our inability to detect IS*3411* insertions into *ureG* in *E. coli* 914 and 187 might indicate that there are other *cis*-acting sequences or undefined host factors involved in targeting the transposition of IS*3411*. There is an IS*3411* element that mapped 5.5 kb away from the urease gene cluster in *E. coli* 1021 (Fig. 5). Possibly it is the close proximity of this element to the urease gene cluster that results in the frequent transposition of IS*3411* into *ureG*.

Urease is an unusual trait in *E. coli*, suggesting that it was introduced into this strain by a mobile genetic element. At the present time, we have no evidence that the urease gene cluster in *E. coli* 1021 is part of a transposon. The apparent association between IS*3411* and urease-encoding *E. coli* strains might be due to cotransfer of IS*3411* and the urease genes via a plasmid or by a transduction event. We have not been able to detect a plasmid in this isolate, suggesting that if the introduction of the trait was plasmid mediated, then the plasmid is now integrated into the chromosome. Alternatively, *E. coli* 1021, 5406, 914, and 187 may have initially originated from the same parent strain and subsequently evolved distinct IS*3411* profiles.

We have previously shown that the *E. coli* 1021 urease genes are encoded on a 4.6-kb DNA fragment. The DNA sequence of 2.5 kb of this fragment was determined; this sequence showed a high degree of similarity to the *K. aerogenes* urease genes *ureA*, *-B*, *-C*, *-E*, *-F*, and *-G*. On the basis of the sequence data the order of urease genes in the two species appears the same. As does the *Klebsiella* urease gene cluster, the *E. coli* 1021 urease gene cluster contains *ureG*. Translation of the region of the *ureG* DNA sequence obtained here into a predicted protein sequence indicated that the *ureG* gene product is highly conserved between the two species. This gene product is mandatory for expression and/or activity of urease in *E. coli*, since insertion of the IS element completely eliminates urease activity. To date the function of *ureG* is unknown.

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