INFECTION AND IMMUNITY, Feb. 1994, p. 606–614
0019-9567/94/$04.00+0

Vol. 62, No. 2

Excision of Large DNA Regions Termed Pathogenicity Islands from tRNA-Specific Loci in the Chromosome of an *Escherichia coli* Wild-Type Pathogen

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Received 21 September 1993/Returned for modification 2 November 1993/Accepted 22 November 1993

Uropathogenic *Escherichia coli* 536 (O6:K15:H31) carries two unstable DNA regions, which were shown to be responsible for virulence. These regions, on which the genes for hemolysin production (*hly*) and P-related fimbriae (*prf*) are located, are termed pathogenicity islands (PAI) I and II, and were mapped to positions 82 and 97, respectively, on the *E. coli* K-12 linkage map. Sequence analysis of the PAI region junction sites revealed sequences of the *leuX* and *selC* loci specific for leucine and selenocysteine tRNAs. The tRNA loci function as the targets for excision events. Northern (RNA) blot analysis revealed that the sites of excision are transcriptionally active in the wild-type strain and that no tRNA-specific transcripts were found in the deletion mutant. The analysis of deletion mutants revealed that the excision of these regions is specific and involves direct repeats of 16 and 18 nucleotides, respectively, on both sides of the deletions. By using DNA long-range mapping techniques, the size of PAI I, located at position 82, was calculated to be 70 kb, while PAI II, mapped at position 97, comprises 190 kb. The excision events described here reflect the dynamics of the *E. coli* chromosome.

Escherichia coli is the most frequent isolate of urinary tract infections. Additionally, cases of newborn meningitis and sepsis have been attributed to *E. coli* pathogens (39). Numerous studies have provided unequivocal evidence that hemolysin production and expression of adherence factors contribute to the pathogenesis of extraintestinal *E. coli* (23, 28, 33). The initial event in colonizing the host tissue is mediated by fimbrial adhesins, which can be distinguished by their receptor specificity (e.g., P, S, and type 1) (13, 14). The hemolytic activity of the pathogenic *E. coli* seems to play a major role during the later stage of the infectious process (17, 33). Spontaneous loss of virulence properties in *E. coli* has been reported previously (15, 26). Genetic data have provided evidence for dynamic events in the *E. coli* genome modulating virulence expression (40).

The *E. coli* uropathogenic isolate 536 (O6:K15:H31) is a well-characterized strain. It has been demonstrated that this strain is hemolytic and produces various types of fimbrial adhesins such as S fimbrial adhesins (*sfa*) and type 1 and P-related fimbriae. Genetic analysis further revealed that two unstable DNA regions carrying the genes for hemolysin (*hly*) production and P-related fimbriae (*prf*) expression are spontaneously deleted from the chromosome of the wild-type strain, leading to mutants with markedly reduced in vivo virulence (15, 17, 26). In this study, we focussed our interest on the size of these unstable regions, termed pathogenicity islands (PAIs).

Additionally, the molecular event of excision is analyzed by sequence studies of the deletion junction sites and by studies on the transcriptional activity at the respective sites. It is shown for the first time that virulence-associated genes are inserted into tRNA-specific loci. The excision of the PAIs may involve repeating sequences as part of the tRNA coding sequences.

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MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Tables 1 and 2. The *E. coli* wild-type strain 536 (O6:K15:H31) was originally isolated from a patient suffering severe pyelonephritis (16). The deletion mutants of this strain were obtained by screening for spontaneous loss of hemolysin production or reduced hemolytic activity (26). The *E. coli* strains were grown on Luria-Bertani (LB) agar or in LB broth. For maintenance of plasmids ampicillin (50 μg/ml) was added. As the recipient in the mating experiments, *E. coli* K-12 AB 519 (*thr leu proA lac trp his argA rpsL metA*) was used; as donors Hfr *E. coli* strains were constructed as described below. As recipients for recombinant DNA *E. coli* K-12 DH5α (*supE44 ΔlacU169 φ80lacZ* ΔM15 *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*) or HB101 (*supE44 hsdS20* r_Bm_B *recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1*) was used.

The cosmids used in this study were based on vector pHC79 and have been described previously (15, 26).

DNA techniques. Isolation of chromosomal and plasmid DNA and recombinant DNA techniques were performed as described by Sambrook et al. (47).

PCR. The PCR was carried out as described by Saiki et al. (46), with a thermocycler 60 apparatus (Biomed, Theres, Germany) and synthetic oligonucleotides obtained from TIBMOLBIOL (Berlin, Germany).

DNA sequencing. DNA sequencing was carried out by the chain-terminating method of Sanger et al. (48), with the T7-sequencing kit from Pharmacia (Freiburg, Germany). For sequencing, double-stranded plasmid DNA or isolated linear DNA fragments and synthetic oligonucleotide primers obtained from TIBMOLBIOL were used. Computer analyses of

TABLE 1. *E. coli* 536 wild type and mutants

Strain	Genotype			Phenotype		Virulence[a]
	PAI I	PAI II		Hly	Prf	
	hly I	*hly* II	*prf*			
Wild-type 536	+	+	+	+	+	+
Mutants						
536-21	−	−	−	−	−	−
536-111	−	−	−	−	−	−
536-112	−	−	−	−	−	−
536-22[b]	−	−	−	−	−	−
536-114	−	+	+	+	+	+[c]
536-225	+	−	−	+	−	−

[a] Estimated following tests of a rat pyelonephritis model (15, 26).
[b] In vivo generated (15).
[c] Moderate virulent (26).

nucleotide sequences were performed with the University of Wisconsin Genetics Computer Group programs of Devereux (8).

PFGE. Genomic DNA for the analysis by pulsed-field gel electrophoresis (PFGE) was prepared and cleaved as described by Grothues and Tümmler (12). Restriction enzymes *Xba*I and *Not*I were obtained from Boehringer (Mannheim, Germany). PFGE was carried out with the CHEF DrII system from Bio-Rad (München, Germany) at 200 V in 0.5× TBE buffer (47) at 12°C (buffer temperature). Pulse times for PFGE are indicated in each of the figure legends. Lambda concatemers, yeast chromosomes (Bio-Rad), and *Hin*dIII-cleaved lambda DNA were used as size markers.

Radioactive labelling and Southern blot analysis. Cleaved DNA separated on agarose gels was transferred to Biodyne nylon membranes as recently described (47). DNA fragments were labelled by the random priming technique, described by Feinberg and Vogelstein (9), by using the kit from Boehringer and [α-P^{32}]dATP obtained from Amersham (Braunschweig, Germany). The DNA probes specific for *hly*, *prf*, *pil*, and *sfa* have been described in detail recently (41). The DNA probes specific for *pyrE* (0.63 kb) and *ilvE* (0.89 kb) were generated by PCR with suitable primer pairs designed according to the sequences published (see reference 3 and references therein). The probe comprising the junction fragment of the deletion I region, HD-2, was generated by PCR as a 387-bp fragment by using a primer pair selected according to sequence data obtained in this study. The probe comprising the junction fragment of deletion II region was cloned as 3.7-kb *Hin*dIII fragment and termed HD-1 (Table 2). Single-stranded oligonucleotides were 5′-phosphate end labelled by using the T4-polynucleotidekinase reaction as described by Ausubel (1). Stringent conditions were used for hybridization; i.e., the hybridization was performed at 42°C in 50% formamide–2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate (SDS), and then 0.1× SSC–0.1% SSC–0.1% SDS was used for washing. For the oligonucleotide probe hybridization, the conditions were 28°C and 20% formamide. Filters were washed twice with 2× SSC–0.1% SDS for 5 min.

TABLE 2. Cosmids and plasmids

Designation	Characteristic(s)	Vector	Reference
pHD-1	3.7-kb *Hin*dIII junction fragment of PAI II of mutant *E. coli* 536-21	pUC 18	This study
pCos 10-21	PAI II, *prf*	pHC 79	26
pCos 1-26	PAI II	pHC 79	26
pCos 15-65	PAI I	pHC 79	26
pH1	PAI II, 17-kb *Hin*dIII right border fragment	pBR 322	This study
pF1	PAI II, 17-kb *Hin*dIII left border fragment	pBR 322	This study
p15-65/2	PAI I, 8-kb *Hin*dIII fragment from pCos 15-65; *selC*	pUC 18	This study
pIE 1041	F6 (IncFI)::Tn*10*		This study

RNA isolation and Northern (RNA) blot analysis. Total RNA of *E. coli* strains was isolated as described previously (4). Northern blot analysis was performed by using 1.5% agarose–formaldehyde gels as described by Ausubel (1). The labelling of the oligonucleotides used as probes and the hybridization were carried out as described above.

Construction of Hfr strains and mating conditions. For mapping the respective regions of the *E. coli* 536 chromosome, several Hfr 536 derivative strains have been constructed. The F6 plasmid (IncFI) tagged by Tn*10* was transferred conjugationally to *E. coli* 536 and to the derivative strains 536-225 and 536-114 (Table 1) by using tetracycline-containing nutrient medium (15 μg/ml) and the F-pilus-specific phage M13 for selecting the corresponding F^+ 536 derivative strains. The obtained exconjugants then were tested for chromosomal marker transfer (e.g., *leu thr his*). Among the F^+ derivatives of strains 536 and 536-114, approximately 10% of the colonies picked and tested displayed a high rate of somatic marker transfer. Analysis of the plasmid profile revealed that the derivative strains did not contain plasmid DNA, an observation that confirmed the integration of the F plasmid (pIE1041) (Table 2) leading to an Hfr status.

For *E. coli* 536-225 derivatives, the transfer frequency of chromosomal markers was generally lower than that for the 536 and 536-114 derivatives and the transfer frequency of the plasmid marker (Tc^+) was higher than that of the somatic markers, indicating an intermediate Hfr status. The mating was carried out as described by Miller (35). The following five Hfr strains were chosen for the experiments described here (Fig. 1): Hfr-1 (536-114) with the transfer origin between *leu* and *pro* in a clockwise manner, Hfr-2 (536-114) with the transfer origin between *thr* and *leu* in a clockwise manner, Hfr-3 (536-114) with the transfer origin between *thr* and *leu* in a counterclockwise manner, Hfr-4 (536-114) with the transfer origin between *met*A and *asp*A in a counterclockwise manner, and Hfr-5 (536) with the transfer origin between *thr* and *leu* in a clockwise manner (Table 3 and Fig. 1). Moreover, Hfr derivatives could not be detected in the F^+ 536-225 strain spontaneously. The F^+ 536-225 strain was therefore used for the mating experiments.

Hemolysin production. Hemolytic activity of *E. coli* strains was analyzed by cultivation of the strains on blood agar plates as described previously (38).

S fimbria- and type 1 fimbria-specific adhesion testing. S fimbrial adhesin expression of *E. coli* strains was analyzed by a hemagglutination assay with plate-grown bacteria. Bovine erythrocytes were used and applied before and after treatment with neuraminidase (16). Type 1 fimbrial expression was determined with *E. coli* grown in static broth. Type 1 fimbria production was tested by agglutination with yeast cells in the presence and absence of 2% D-mannose (16).

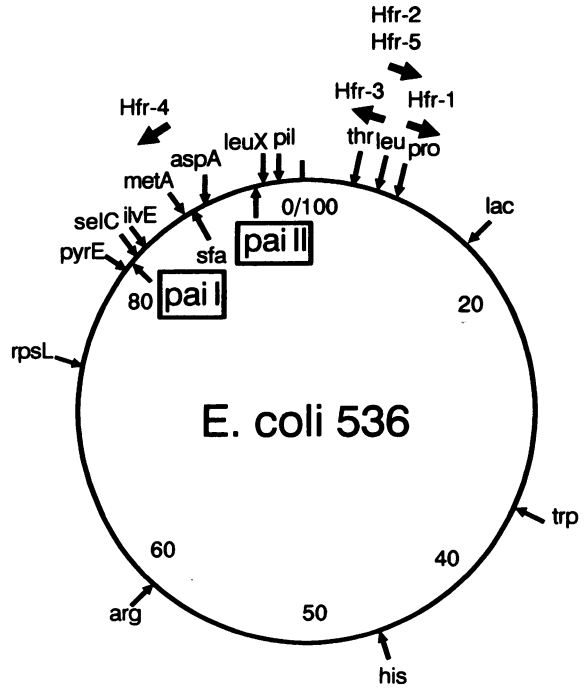

FIG. 1. Map positions of the pathogenicity islands of *E. coli* 536, according to the *E. coli* K-12 linkage system (3). The locations of origins of transfer of the respective Hfr strains are further indicated in Table 3.

RESULTS

PAI I and PAI II are located at tRNA loci. Deletion mutant *E. coli* 536-21, which has lost both PAIs, has been previously described by Knapp et al. (26) and Hacker et al. (15) (Table 1). The junction fragments of the PAI I region had been cloned and partially sequenced. As shown previously, the deleted region of PAI I is flanked by 16-nucleotide (nt) direct repeats: 5′-**TTCGA**CTCCTGTGATC-3′ (Fig. 2A) (boldface type indicates motif shared by both PAIs). Upon deletion of the 70-kb region, a single repeat remains at the excision site. Further sequence analyses at the deletion site were carried out (Fig. 2A). It was shown that the right-handed repeat is part of the complete sequence encoding tRNA for selenocysteine (*selC*) (29), which is disrupted by the deletion process.

In a similar manner, deletion site II has been cloned from a genomic library of *E. coli* 536-21, by using the right and left end points of PAI II as DNA probes (data not shown). A 3.7-kb *Hin*dIII fragment of strain 536-21 was shown to contain the junction of the deleted region. This fragment was cloned into pUC 18 to obtain plasmid pHD-1 (Table 2). Restriction enzyme mapping and sequence analysis of this fragment and the cloned right and left endpoints (pH1 and pF1, respectively) (Table 2) revealed that an 18-nucleotide (nt) direct repeat, 5′-GTTCGAGTCCGGCCTTCG-3′, flanks the excision site (Fig. 2B). As with the PAI I deletion, one of the repeats remains at the excision site after the deletion event. Furthermore, it has become evident that the right-handed deletion site comprises the sequence encoding the complete tRNA for leucine (*leuX*) (45, 50), which is disrupted by the deletion. The repeating sequences at PAI I and PAI II share the motif -TTCGA-, which is part of the conserved tRNA loops (see above).

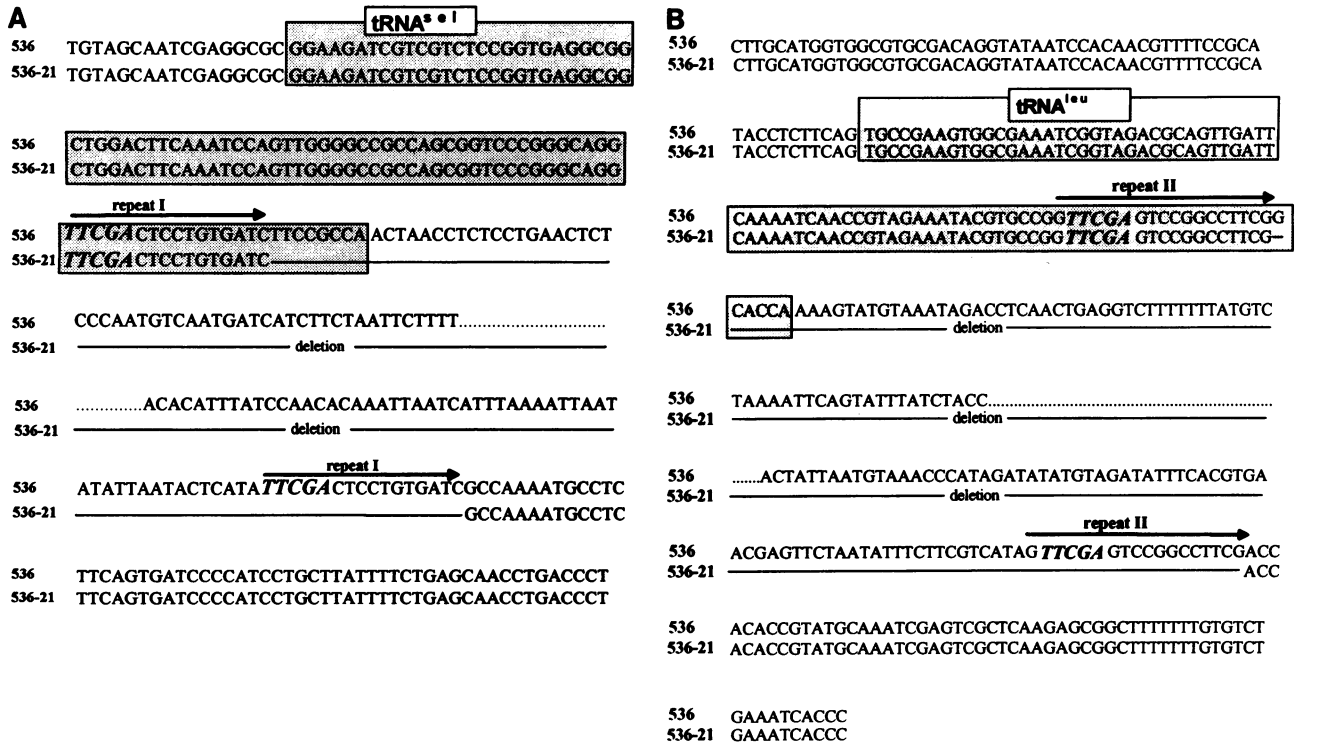

FIG. 2. Nucleotide sequences at the deletion sites of PAI I (A) and PAI II (B). The tRNA coding sequences *selC* and *leuX* are shown in shaded boxes. Boldface italics indicate the shared motif.

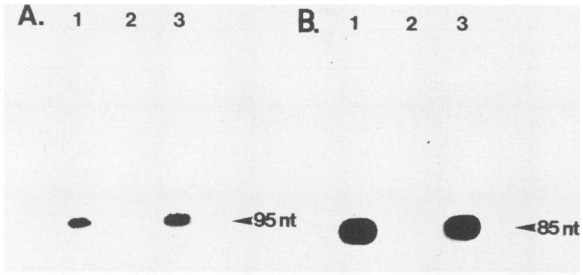

FIG. 3. Northern blot analysis of total RNA of *E. coli* 536 (lanes 1), 536-21 (lanes 2), and HB 101 (lanes 3) by using the antisense repeats of PAI I (A) and PAI II (B) as probes. The sizes of RNA transcripts (arrowheads) are 95 nt (A) and 85 nt (B).

Analysis of the transcriptional activity of the tRNA loci. In order to determine whether the tRNA genes of the wild type and mutant are transcribed, antisense repeats I and II were used in Northern blot analysis of total RNA isolated from *E. coli* 536, the deletion mutant 536-21, and *E. coli* K-12 HB 101 (Fig. 3). It was observed that the wild-type RNA hybridizes to repeat I in a 95-nt transcript and to repeat II in an 85-nt transcript (lanes 1). Additionally, in *E. coli* K-12 HB 101, transcripts of the same sizes were detected (lanes 3), whereas RNA of the deletion mutant 536-21 displayed no hybridization to the site I or II repeat probes (lanes 2). These findings indicate that upon deletion, transcription of the respective tRNAs (*selC* and *leuX*) is abolished.

Size determination of PAI I and II. In order to determine the sizes of the PAI regions and to gain insight into the genome structure following the deletion events, *E. coli* 536 wild-type DNA and genomic DNA of the deletion mutant 536-21 were analyzed by PFGE after cleavage with *Xba*I (Fig. 4). Additionally, Southern hybridization was performed by using fragments specific for S fimbrial adhesins (*sfa*), P fimbrial adhesins (*prf*), hemolysin (*hly*), type 1 fimbriae (*pil*), and the junction fragments HD-1 (PAI II) and HD-2 (PAI I) as DNA probes.

It was demonstrated that two fragments with sizes of 370 and 50 kb reacted with the *hly* probe in the wild-type strain (Fig. 4B, lane 1), while the *prf* probe hybridized only in the 370-kb fragment (Fig. 4C, lane 1), indicating that the 370-kb fragment is PAI II specific (15). No hybridization occurred in the mutant genome with the *hly* and *prf* probes (Fig. 4B and C, lanes 2). With an *sfa*-specific probe, a 470-kb *Xba*I fragment could be detected in both strains (Fig. 4E, lanes 1,2), whereas the wild type and the mutant reacted with a *pil* probe in a 320-kb fragment (Fig. 4D, lane 1) and a 500-kb fragment (Fig. 4D, lane 2), respectively. By using the HD-1 fragment (specific for the PAI II junction site) as the probe, 370- and 320-kb fragments were detected in the wild-type strain (Fig. 4F, lane 1). A 500-kb fragment was observed in the deletion mutant (Fig. 4F, lane 2). From these data it can be concluded that the 370-kb (carrying the physically linked *hly* II and *prf* genes (15) and 320-kb (carrying the *pil* genes) fragments lie adjacent to each other in the wild-type genome. Upon deletion of PAI II, a 500-kb fragment carrying the *pil* genes is generated. By adding the fragment sizes 370 and 320 kb and subtracting the size of the newly generated fragment of 500 kb, the PAI II region can be calculated to a size of 190 kb, which contains the *hly* II and *prf* genes. The deletion does not affect the *sfa* and *pil* loci but occurs in the vicinity of the *pil* locus.

By using the junction fragment of PAI I, HD-2 (see Materials and Methods), the wild-type genome displays hybridization in two fragments with sizes of 110 and 290 kb (Fig. 4G, lane 1), while the mutant hybridizes in a 380-kb fragment (lane 2). Since the *hly* I-specific fragment is 50 kb in size (Fig. 4B, lane 1), the fragments detected by the junction probe must flank the 50-kb fragment (see Fig. 7). By adding the sizes of the three fragments, 290, 50, and 110 kb, and subtracting the size of the newly generated 380-kb fragment in the deletion mutant, the size of the PAI I region can be calculated to be 70 kb. This is well supported by the data obtained through chromosome walking (26).

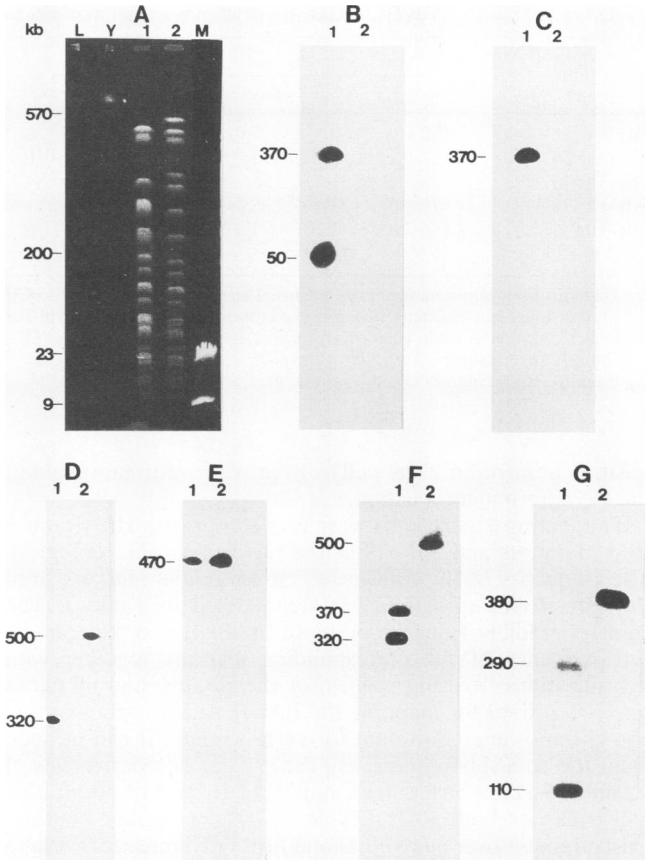

FIG. 4. Genomic *Xba*I pattern of *E. coli* 536 wild-type (lanes 1) and mutant 536-21 (lanes 2) obtained by PFGE (A) and Southern hybridization with *hly* (B)-, *prf* (C)-, *pil* (D)-, and *sfa* (E)-specific fragments and the cloned junction fragments HD-1 of *hly* II (F) and HD-2 of *hly* I (G) as DNA probes. In panel A, yeast chromosomes (lane Y), lambda concatemers (lane L), and *Hin*dIII cleaved lambda DNA (lane M) were used as DNA size markers. Electrophoresis was carried out with pulse times increasing from 5 to 40 s over a period of 24 h.

Mapping of PAI II and the *sfa* genes on the chromosome of *E. coli* 536. The data from the DNA long-range mapping indicate that the *hly* II gene is in the vicinity of the genes encoding type 1 fimbriae (*pil*) (see above). The deletion event leads to an alteration of the *Xba*I hybridization pattern by using the *pil* probe, and the same alteration of the *Xba*I fragments was detected by using the PAI II junction fragment (HD-1) as a DNA probe. The *pil* locus is mapped to 98 units in the *E. coli* K-12 linkage map (3). This indicates the location of PAI II to be at that region in the chromosome of *E. coli* 536. This is further corroborated by the finding that the leucine tRNA gene (*leuX*), which is mapped according to Bachmann at position 97, is the target for the excision. Further, mating experiments were undertaken to confirm the PAI II proposed

TABLE 3. Transfer of chromosomal markers from *E. coli* 536 Hfr derivative strains to *E. coli* AB 519

Strain	Cotransfer frequency[a]													
	thr	*leu*	*proA*	*lac*	*trp*	*his*	*gyrA*	*argA*	*rpsL*	*hly* I[b]	*metA*	*sfa*[b]	*hly* II[b]	*pil*[b]
Hfr-1 (536-114)	182	180	1	0	0	0	1	0	0	0	13	10	35	108
Hfr-2 (536-114)	157	3	1	2	0	0	0	0	2	0	5	17	69	86
Hfr-3 (536-114)	3	121	86	63	31	14	5	3	3	0	0	0	0	0
Hfr-4 (536-114)	76[c]	73	37	21	1	1	1	0	0	0	0	76[c]	76[c]	76[c]
Hfr-5 (536)	136	1	1	1	0	0	0	0	1	0	9	6	61	72
F^+ 536-225	12[d]	12	6	7	2	1	3	1[e]	5	3[d,e]	3	NT	0	3[d]

[a] Data are from representative experiments. The numbers correspond to CFU in 100 μl of conjugation mixture after 2 h of incubation at 37°C. NT, not tested.
[b] Marker frequency estimated according to cotransfer by using the corresponding gene probes (see the text).
[c] Only *thr*$^+$ colonies could be checked for *sfa*, *pil*, and *hly*.
[d] From 12 *thr*$^+$ colonies, 3 were *hly*$^+$ and *pil*$^+$.
[e] From five *arg*$^+$ colonies tested, only one gave a positive signal to *hly*.

location at position 97 as well as to provide information about the *sfa* determinant location.

The mating experiments were carried out with Hfr strains 1 to 5 as donors and AB 519 as the recipient (Table 1; Fig. 1). The frequency of the transferred chromosomal markers from the Hfr strains to AB 519 is summarized in Table 3. The transfer gradient from the origin to the PAIs and the cotransfer frequency of the corresponding markers together with hybridizations by using probes for the *sfa*, *hly*, and *pil* genes have been used for mapping the PAI II and *sfa* genes on the 536 chromosome equivalent to the K-12 map. It can be seen from the transfer frequencies (Table 3) of Hfr derivatives of strain 536-114, which carries only PAI II, and of strain 536, which carries both PAIs (Table 1), that a marker gradient exists from *thr* over *pil* to *hly* II and further counterclockwise to *sfa* and *met*A. This implies the location of PAI II to be in the vicinity of *pil* while suggesting the *sfa* determinant to be located at approximately position 92 in the vicinity of *met*A (position 91). The data obtained with the Hfr-4 strain confirm these locations, since a high cotransfer frequency of *sfa*, *hly*, and *pil* with *thr* but not with the *met*A marker was observed. The position of the PAI II region determined by the mating experiments is in agreement with the results obtained by DNA long-range mapping (see above) (Fig. 1).

Mapping of PAI I on the chromosome of *E. coli* 536. As shown for the above mating experiments to map PAI II and the *sfa* locus, F^+ strain 536-225 (Table 3), a derivative of strain 536-225, carrying only PAI I (see Table 1) was used for mapping PAI I. As can be seen from the data in Table 3, the transfer frequencies are low compared with those of the Hfr strains. This is due to the fact that F^+ strain 536-225 represents only an intermediate Hfr status (see Materials and Methods). From the marker cotransfer frequencies it can be concluded that the PAI I region must be located between *rpsL* (73 units) and *metA* (91 units). Since further suitable markers are not available, a precise location of PAI I by mating experiments cannot be determined.

Since the selenocystyl tRNA (*selC*) gene was mapped to position 82.3 on the *E. coli* K-12 linkage map, it was concluded that PAI I, whose deletion coincides with the disruption of the *selC* gene (see above), is located at that position. To provide further information on the location of PAI I, DNA probes specific for *pyrE* and *ilvE* were generated. The map position of *ilvE* is 82.6 and that of *pyr* E is 82.0 (3). *Not*I-cleaved genomic DNA of the *E. coli* 536 wild type and the mutant 536-21 was hybridized to the repeat I, the *hly*-, *pyrE*-, and *ilvE*-specific gene probes. All four probes used hybridized to the same 330-kb fragment of the wild-type DNA (Fig. 5B to E, lanes 1), while in

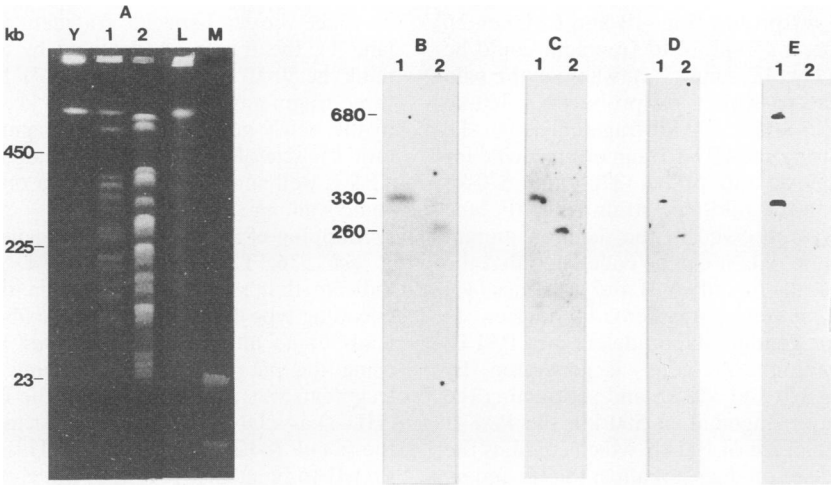

FIG. 5. Genomic *Not*I pattern of *E. coli* 536 wild-type (lanes 1) and mutant 536-21 (lanes 2) obtained by PFGE (A) and Southern hybridization by using repeat I (*selC*) (B)-, *pyrE* (C)-, *ilvE* (D)-, and *hly* (E)-specific fragments as DNA probes. In panel A, yeast chromosomes (lane Y), lambda ladders (lane L) and *Hin*dIII cleaved lambda DNA (lane M) were used as size markers. Electrophoresis was carried out with pulse times increasing from 5 to 50 s over a period of 24 h.

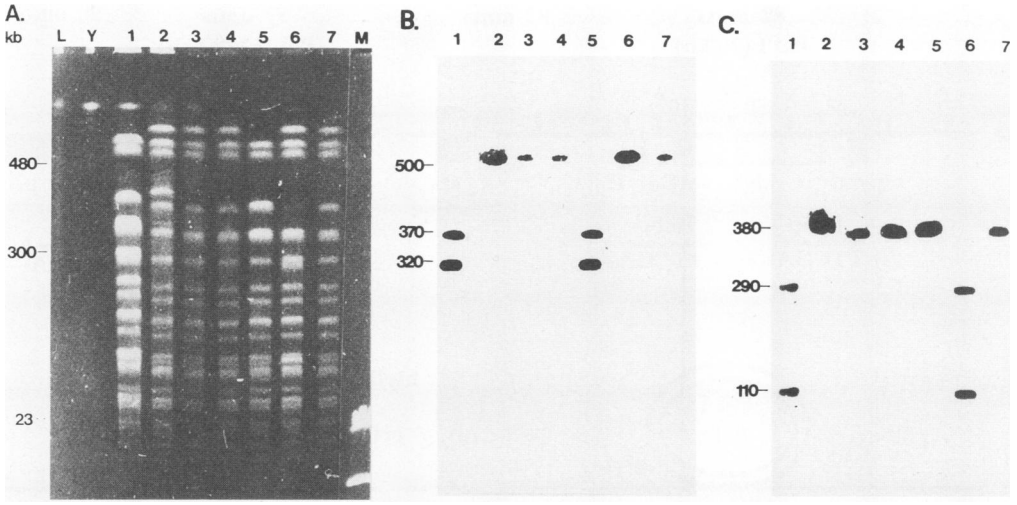

FIG. 6. *Xba*I genomic profile of *E. coli* 536 wild-type and mutant strains (Table 1) obtained by PFGE (A) and Southern hybridization to the junction fragments of the unstable *hly*II region HD-1 (B) and of the unstable *hly* I region HD-2 (C). Lanes: 1, strain 536; 2, 536-21; 3, 536-111; 4, 536-112; 5, 536-114; 6, 536-225; 7, 536-22. In panel A, yeast chromosomes (lane Y), lambda concatemers (lane L), and *Hin*dIII cleaved lambda DNA (lane M) were used as size markers. Electrophoresis was carried out with pulse times increasing from 5 to 40 s over a period of 24 h.

the mutant hybridization of the repeat I, *pyrE*, and *ilvE* probes to a 260-kb fragment was found (Fig. 5B to D, lanes 2). The wild-type DNA (Fig. 5E, lane 1) shows an additional band of 680 kb with the *hly*-specific probe, which corresponds to the PAI II region. These data show that the PAI I region is located in the vicinity of the *pyrE* and *ilvE* markers, and thus it is concluded that the map position is 82 units. A map of the *E. coli* 536 chromosome is depicted in Fig. 1.

Analysis of the specificity of the deletion events in various mutants of *E. coli* 536. To prove that the deletion is a specific event, we analyzed various mutants (Table 1) by hybridizing *Xba*I-cleaved genomic DNA separated by PFGE to the junction fragments HD-1 and HD-2 of the PAI II and PAI I regions. It can be seen in Fig. 6A that those mutants which had lost both regions (lanes 2, 3, 4, and 7) show an identical *Xba*I pattern, which is distinguishable from the pattern of the mutants that have lost one of the two regions (lanes 5 and 6) and that also differ from each other as well as from the pattern of the wild type (lane 1). By comparing the four different patterns, *Xba*I fragments specific for PAI I and PAI II deletions can be determined. Hybridization to the PAI II junction fragment, HD-1 (Fig. 6B), revealed that those mutants which had lost either PAI II (lane 6) or both PAIs (lanes 2, 3, 4, and 7) hybridized to a 500-kb *Xba*I fragment, whereas the wild type and the mutant with deletion of PAI I displayed two fragments with sizes of 320 and 370 kb (lanes 1 and 5). By using the PAI I junction fragment, HD-2 (Fig. 6C), as the DNA probe, the mutant with the PAI I deletion (lane 5) showed the same pattern as the mutants which have lost both PAIs (lanes 2, 3, 4, and 7), all hybridizing in a 380-kb fragment. The wild type (lane 1) and the mutant with deletion of PAI II (lane 6) display hybridization in 110- and 290-kb fragments. From these data it can be concluded that such deletions are independently and specifically occurring in vitro as well as in vivo, since mutant 536-22 was isolated from the rat kidney after inoculation with *E. coli* 536 wild-type bacteria (15).

DISCUSSION

E. coli 536 is a uropathogenic isolate which carries two large DNA regions, termed PAI I and PAI II, in its chromosome. The genes coding for hemolysin (*hly*) and P-related fimbriae (*prf*) are located on PAI I and II. These genes together with *prf* regulatory sequences which activate the S-fimbrial-specific genes (36) are necessary for full virulence of strain 536. Consequently, deletion mutants which have lost PAIs I and II also show a decreased virulence potency (15). In this study the sites of the deletion of the PAIs were analyzed in detail. DNA sequence analysis revealed that the genes for tRNAs which are disrupted by the deletion process are located at both sites.

As shown in Fig. 7, the PAIs could be mapped to position 82 (PAI I) where the tRNA for selenocysteine (*selC*) is located and to position 97 (PAI II) at the position of the leucine tRNA gene (*leuX*). Direct repeats of 16 and 18 nt flank PAI I and PAI II, respectively. The repeats share the motif -TTCGA-, which is part of the conserved tRNA loop. The excision might occur by recombinational events involving the repeats. Future studies using *recA* mutant derivatives of *E. coli* 536 will be helpful in determining the influence of the RecA protein on the instability of the PAIs. Additionally, transcriptional activity is observed at either of the tRNA loci of the wild-type strain, which is abolished after excision of the PAIs. Furthermore, a pronounced clustering of insertion elements found at position 97 in *E. coli* K-12 (6) may also influence the deletion of PAI II.

It has been shown that retronphages carry parts of tRNA genes (21) and contribute to the instability of genomic sites in bacteria. It might be speculated that also in the case of the *E. coli* 536 genome instability, such elements are involved in the excision processes. Interestingly, tRNA genes are frequently used as integration sites of plasmids and phages in various bacteria (19, 43). This might lead to the conclusion that the PAIs of *E. coli* 536 might have their origin in plasmids. Plasmids carrying hemolysin determinants have been described previously (16), and integrations of plasmids into the bacterial chromosome are well-known phenomena (40, 51), a process for which an involvement of insertion elements has been described (7, 42). On the other hand, excisions of integrated plasmid sequences from the bacterial chromosome have been observed (5, 27). Studies that are presently under way for detecting intermediate products of the excised Pais of *E. coli* 536 will provide information on the fate of deleted regions, i.e.,

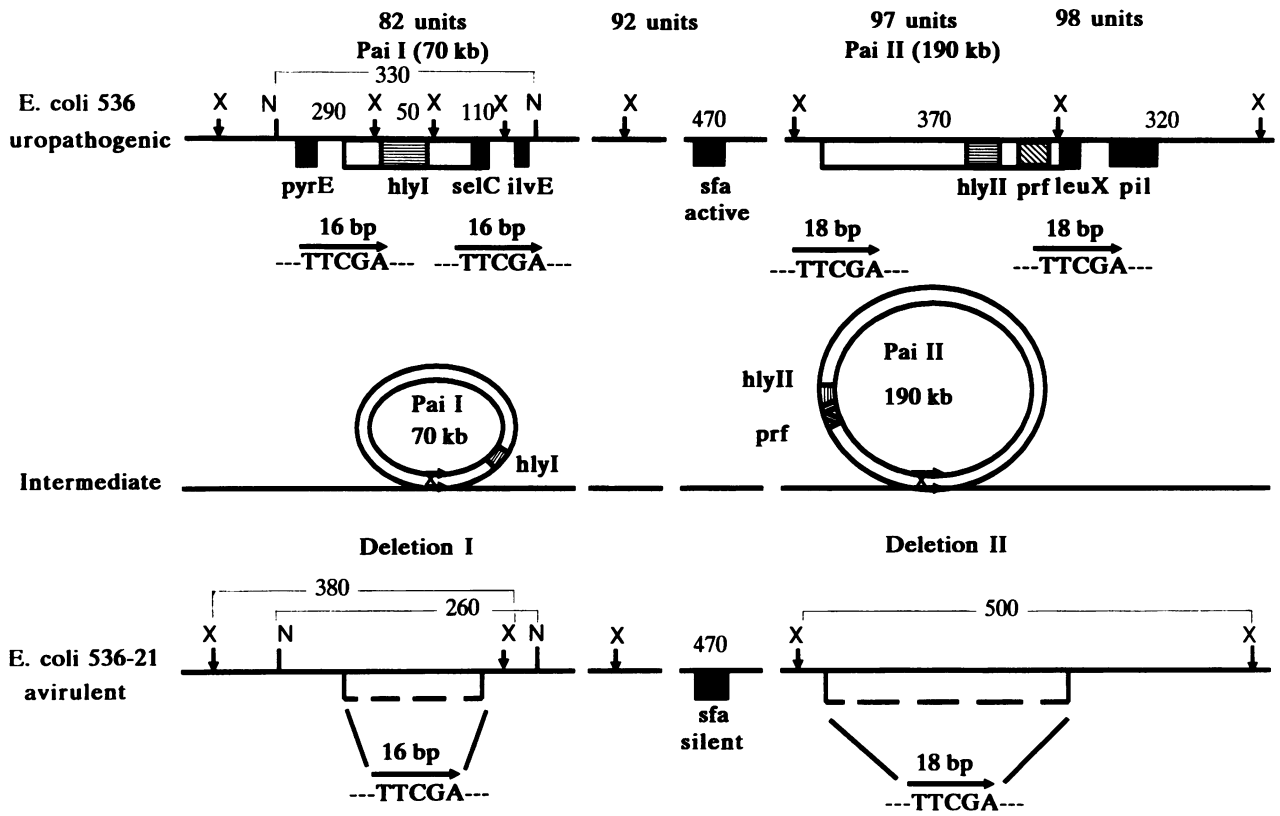

FIG. 7. Model for the excision of PAI I and PAI II from the chromosome of *E. coli* 536. Putative recombinational events and intermediate products are indicated. For the PAI I and PAI II regions, *Xba*I sites are indicated by X and *Not*I sites are indicated by N. The sizes of the fragments are given in kilobases (see the text).

whether there is a transferability of PAIs to other *E. coli* strains. These investigations may provide insights into the evolution of *E. coli* pathogens.

The physical linkage of hemolysin and P-fimbrial gene cluster on the one hand (20, 31) and the instability of the genome of *E. coli* on the other seem to be a more general phenomenon, especially with the deletion of virulence-associated characters observed in other *E. coli* strains of extraintestinal source (15). This kind of dynamics of the genome might have biological implications. A smaller genome lowers the generation time of *E. coli*, and the irreversible loss of fimbriae and hemolysin genes also could be an advantage in the later stage of urinary tract infection, by circumventing the host immune response. In this respect it is noteworthy that *E. coli* strains isolated from impaired patients exhibit fewer virulence-associated traits, compared with strains isolated from noncompromised patients (24). Interestingly, as for the mutants of *E. coli* 536, the genes for hemolysin production and P fimbriae were missing. Furthermore, there is a body of evidence provided by epidemiological data that nonvirulent strains can be isolated from urine samples from patients with chronic urinary tract infections more often than from patients with acute urinary tract infections. The longer the patient suffers, the higher the number of strains devoid of virulence factors detected (11, 37). Molecular processes similar to that described for the uropathogenic *E. coli* strain 536 in this study might have been the basis for the evolvement of such strains.

The disruption of the *selC* gene following the deletion process leads to mutants which no longer express selenocysteine tRNA. Consequently, proteins with selenocysteines should be nonfunctional. The formate dehydrogenase which decomposes formate to carbon dioxide and molecular hydrogen in the mixed-acid fermentation of enterobacteria is one of the *E. coli* proteins with a selenocysteine in its amino acid sequence (2, 30, 44). The objective of future studies will be the demonstration of the lack of formate dehydrogenase activity of PAI I deletion mutants.

A deletion event at PAI I resulting in formate dehydrogenase-negative strains would lead to an accumulation of formate, which lowers the cytoplasmatic pH, negatively affecting the viability of the *E. coli* cell. Besides the loss of the virulence genes, the hereby reduced fitness of the deletion mutants could be one explanation for the decreased in vivo virulence. In contrast, the disruption of *leuX* by excision of PAI II is presumed to have no profound negative effect on the viability of the deletion derivatives, since multiple genes encoding leucyl-tRNAs are present in the *E. coli* chromosome (45, 50).

Processes similar to the deletion events of uropathogenic *E. coli* have been described for other bacterial species. *Streptomyces* spp. show a high plasticicity of the genome. Deletions of large chromosomal regions and integrations of plasmids have been described previously (25, 27, 49). In addition, in the bacterial pathogen *Yersinia pestis*, spontaneous deletions of large DNA regions from the chromosome have been observed (22, 32). Fetherson et al. (10) demonstrated that the loss of 102 kb of chromosomal DNA leads to nonpigmented yersiniae. At both ends of the deletion, repetitive elements of 2.2 kb in size found in direct orientation were considered to be IS*100* and responsible for generating the deletion. The pigmentation phenotype (Pgm) encompasses a variety of traits that are

missing in the Pgm$^-$ mutants, including the production of iron-repressible outer membrane proteins (Irp), which are involved in virulence expression of yersiniae. Deletions of DNA regions altering or abolishing the expression of virulence factors have been reported for numerous other bacterial pathogens (e.g., *Haemophilus influenzae*, *Streptococcus pyogenes*, and others) (for a review, see reference 40); however, presently, the deletions described in this study and the above-mentioned deletions in *Y. pestis* are the only known examples in which such large chromosomal regions have been lost.

Virulence modulation due to dynamic events may contribute to the adaptation of bacterial pathogens at certain stages of the infectious process and may also be of relevance for bacterial survival in the environment. Coordinate regulation of virulence expression by reversible "on" and "off" switching virulence characters has been described for numerous pathogens (34). Conversely, the deletion process described here is an irreversible event, which might be seen as an additional mode of virulence modulation.

ACKNOWLEDGMENTS

We thank L. R. E. Phillips (Würzburg, Germany) for helpful discussions and critical reading of the manuscript.

The work was supported by the Deutsche Forschungsgemeinschaft and the Bundesministerium für Forschung und Technologie (DFG grants Ha 1434/1-7 and Ha/BEO:III/4372, Graduiertenkolleg "Infektiologie") and the Fonds der Chemischen Industrie.

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