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An invertible element of DNA controls phase variation of type 1 fimbriae of *Escherichia coli*

(genomic rearrangement/transcription/operon fusion)

JOHN M. ABRAHAM*, CYNTHIA S. FREITAG*, JANICE R. CLEMENTS*, AND BARRY I. EISENSTEIN*†‡

Departments of *Microbiology and †Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78284

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ABSTRACT **The expression of type 1 fimbriae (pili) of *Escherichia coli* is turned on and off at the transcriptional level at a high frequency (10^{-3} per cell per generation) in a process termed phase variation. Using Southern blot and DNA sequence analysis, we have detected a genomic rearrangement in the switch region immediately upstream of the fimbrial structural gene. This rearrangement involves an invertible 314-base-pair segment of DNA whose alternating orientation apparently results in the on-and-off activation of a promoter that determines the state of fimbrial expression.**

Type 1 fimbriae (pili) are major surface appendages that mediate binding of *Escherichia coli* to eukaryotic cells by a ligand–receptor mechanism that is sensitive to the presence of mannose. The fimbrial protein adhesin is thought to be a virulence factor during the initial colonization stage. During the invasive stage it would seem to be to the pathogen's advantage not to express adhesins that could mediate binding to phagocytic cells. A genetic regulatory system that would result in an on-and-off expression of an adhesin would itself be a virulence factor (1).

The phase variation between fimbriate (Fim$^+$) and nonfimbriate (Fim$^-$) *E. coli* cells occurs at the transcriptional level (2). In addition, it has been shown that a *cis*-dominant DNA switch is turned on and off by means of a *trans*-active factor (3) different from that reported by Orndorff and Falkow (4). Here we report that the molecular basis of this switch is the inversion of a relatively small segment of DNA that results in the alternating activation of the fimbrial promoter in correlation with phase variation. This system resembles in general that which controls flagellar phase variation in *Salmonella* (5) but differs both in its recombinase specificity and in its small size, so that the element does not encode its own recombinase.

MATERIALS AND METHODS

Bacterial Strains and Media. *E. coli* K-12 strain CSH50 [*ara* Δ(*lac–pro*) *rpsL thi*] was the parental strain used. The genetic construction of strain VL412 and bacteriophage λ412 has been presented in detail (3). 5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal) indicator plates and all other routine media were prepared as stated previously (3). The Fim phenotype was determined by a yeast agglutination test (6) and a bacterial agglutination test utilizing a fimbria-specific monoclonal antibody (7).

DNA Manipulations. Recombinant DNA techniques, preparation of the DNA probes, and high-stringency Southern blotting were performed as described by Maniatis *et al.* (8). Restriction enzyme digestions were conducted as suggested by the vendor (New England Biolabs, Bethesda Research Laboratories). For nucleotide sequence determination, the Sanger dideoxy sequencing method was used (9).

RESULTS

The Lac$^-$ *E. coli* K-12 strain CSH50, used in the genetic constructions outlined below, undergoes fimbrial phase variation. Eisenstein (2) described the construction of a *fim-lac* operon fusion in this strain and used its oscillating Lac$^+$⇄Lac$^-$ phenotype to show that phase variation is under transcriptional control (2). The *fim*$^+$, *fim-lac* merodiploid strain VL412 (Fig. 1) was constructed through integration of a specialized λ phage, carrying the *fim-lac* operon fusion, by homologous recombination with the *fimD* gene (3). This merodiploid strain exhibits independent, reversible, and noncoordinated phase variation of both the Lac and Fim phenotypes, which indicates the presence of two *cis*-acting switches, each adjacent to its respective operon (3). Ultraviolet induction of VL412 yields the phage λ412, which alternates between a very dark blue (Dk) and light blue (Lt) plaque phenotype when plated on strain CSH50 on β-galactosidase indicator medium. The switching frequency of this reversible Lac$^+$⇄Lac$^-$ expression is $\approx 10^{-3}$ per phage per lytic cycle and is not the result of sequential DNA deletion (3). Thus the *cis*-dominant switch has been inserted *in vivo* into the genome of the specialized λ phage. All of the CSH50 DNA carried on λ412 was subcloned in pBR322 to yield pJLA2, which was further subcloned in phage M13 vectors to yield templates for use in DNA sequencing and Southern blot analysis.

We have determined the nucleotide sequence of the switch region and downstream DNA carried on phage λ412 and found that it is in good agreement with that reported by Klemm (10) for the fimbrial structural gene and its upstream DNA. Thus, the *lacZ* insertion is in the fimbrial structural gene. The *cis*-acting switch regulates the fimbrial gene itself rather than directly controlling adjacent (e.g., assembly) genes required for organelle expression.

DNA rearrangements in the Dk⇄Lt switching of the specialized phage were detected with Southern blot analyses using a spectrum of restriction enzymes. The DNA probe used to explore the Dk⇄Lt switching λ phage is represented in Fig. 2*a*. The positions of the restriction enzyme cleavage sites shown relative to the structural gene are those first published by Klemm (10) and are now known to represent the "off" orientation of the invertible element (see below). This probe includes the first 140 base pairs (bp) of the fimbrial subunit structural gene and extends upstream ≈1500 bp. A Dk λ phage lysate (Fig. 2*b*, lanes 1 and 3) and a Lt λ phage lysate (lanes 2 and 4) were each used to prepare DNA for digestion and hybridization analysis. (It should be noted that although the Lt DNA preparations were ≥99% pure, the Dk

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Abbreviations: Dk, dark-plaque phenotype; Lt, light-plaque phenotype; bp, base pair(s).

‡To whom reprint requests should be addressed.

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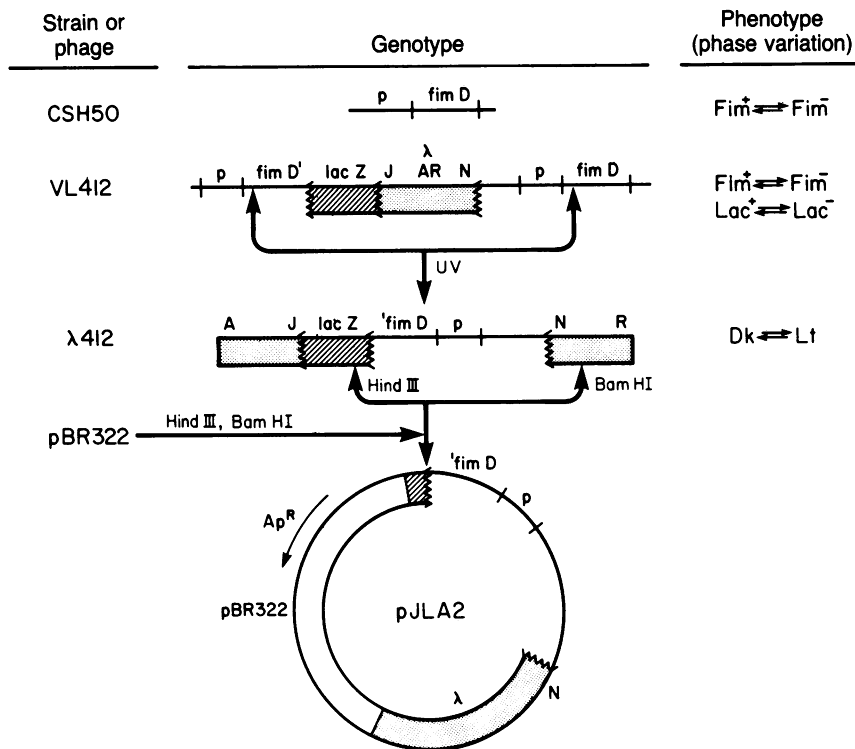

FIG. 1. The genetic construction of the *fimD'-lac* operon fusion and its cloning in pBR322. All of the CSH50 DNA present on the specialized λ transducing phage was inserted into pBR322. Thin lines represent CSH50 DNA, open bars represent pBR322, cross-hatched regions represent *lac* DNA, and stippled bars represent λ DNA. Ap^R, ampicillin-resistance gene; p, promoter sequence; A, J, N, and R, individual λ genes.

preparations were contaminated with approximately 10% of the Lt phage. These relative-purity percentages were very reproducible with multiple large-scale preparations of λ phage lysates. This apparent unequal reversion rate is due to a more rapid propagation of λ phage that are in the Lac^- phase.) After digestion with either *Hin*fI or *Tha* I, differences in hybridization patterns between the Dk and Lt λ DNAs were observed under high-stringency hybridization and wash conditions. The sizes of the hybridizing bands unique to each phenotype are summarized in Table 1. In addition, in both the *Hin*fI and *Tha* I digests, the combined sizes of the fragments unique to the Dk phage were approximately the same as those of the Lt fragments, which would be expected with an invertible segment of DNA.

To show that this rearrangement occurs in the chromosome, genomic DNA was isolated from four alternating phenotypic populations of fimbriate or nonfimbriate CSH50 bacteria. Starting with Fim^+, each successive phase-type was obtained from a phase-switched cell present in the preceding population. We found that genome rearrangement was associated with phase variation; *Hin*fI digestion of these preparations resulted in hybridization of the probe to an 1850-bp band in both Fim^+ phenotypes that was replaced in the Fim^- phenotypes by a 1575-bp band (Fig. 2*b*). Further analysis was confounded by the presence of multiple copies, in the *E. coli* genome, of the DNA sequences found upstream of the *fimD* gene, which resulted in the appearance of multiple hybridization bands. This indicates that at least some of the DNA sequences found in the probe are present in multiple copies in the genome. Nevertheless, it was possible to detect in the $Fim^+ \rightarrow Fim^-$ preparations the loss of a large band and the appearance of two new bands (Fig. 2*b*, lanes 6 and 8).

To determine the on-or-off orientation of the published DNA sequence (10) of the phase-variation switch, a probe consisting only of *fimD* structural gene DNA that is downstream from the invertible segment was used in Southern blot analysis of the Dk and Lt phage DNAs. This probe detected only the larger (1600-bp) fragment in the *Hin*fI digest of the

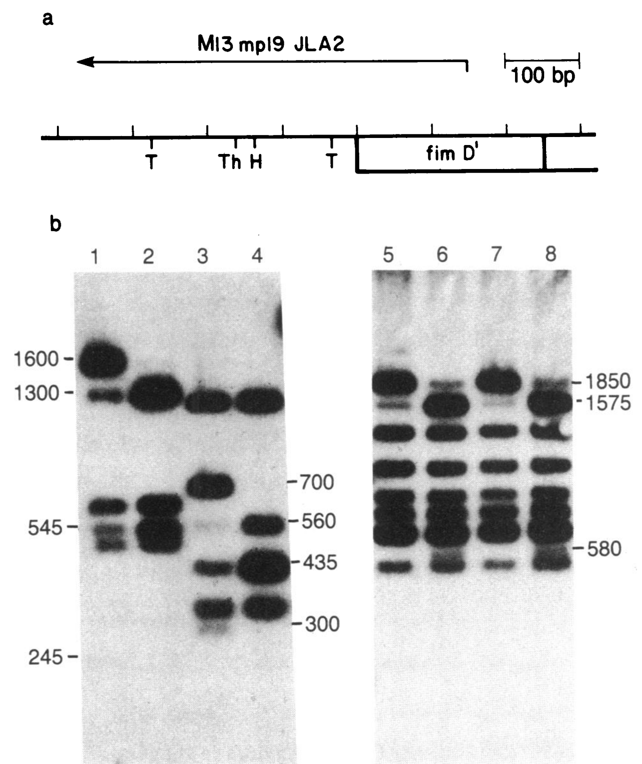

FIG. 2. (*a*) Schematic representation of the *fimD'-lac* operon fusion and the probe used in Southern blot analysis. Phage M13mp19JLA2 begins 140 bp inside the structural gene and then extends upstream for ≈1500 bp. Restriction sites shown are *Taq* I (T), *Tha* I (Th), and *Hin*fI (H). (*b*) Southern blot analysis of DNA using the probe M13mp19JLA2. Lanes: 1 and 3, Dk λ DNA; 2 and 4, Lt λ DNA; 5–8, *E. coli* $Fim^+ \rightarrow Fim^- \rightarrow Fim^+ \rightarrow Fim^-$ DNA, respectively. Lanes 1, 2, 5, 6, 7, and 8 contain DNA digested with *Hin*fI; 3 and 4, DNA digested with *Tha* I. Size markers are in bp.

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Table 1. Sizes (in bp) of the hybridizing fragments unique to each phenotype of the on and off expression states

	λ DNA				*E. coli* DNA	
Enzyme:	*Hin*fI		*Tha* I		*Hin*fI	
Phenotype:	Dk	Lt	Dk	Lt	Fim⁺	Fim⁻
	1600	1300	700*	435	1850	1575
	245	545	300*	560	300†	580

DNA preparations were digested with the indicated restriction endonuclease and analyzed with probe M13mp19JLA2 in Southern blot hybridizations.

*The 700-bp *Tha* I fragment yields a 435-bp fragment and the 300-bp fragment gains ≈260 bp to yield a 560-bp fragment.

†This 300-bp band is not visible in Fig. 2*b*, lanes 5 and 7, but is evident after a longer exposure time.

Dk DNA and only the larger (1300-bp) fragment in the Lt DNA (data not shown). Based on the published position of the *Hin*fI site, which agrees with our sequence data, we conclude that the DNA sequence previously published represented DNA in the off orientation (10). The Southern blot analysis offers good evidence of a DNA inversion upstream of the structural gene but does not absolutely prove it or define the exact size of the DNA inversion. Therefore, we next determined the DNA sequence of the inversion region in both the on and off orientations. The DNA sequence of the inversion element in the on orientation (Fig. 3*a*) was obtained by the Sanger dideoxy chain-termination method. This DNA was cloned from the Dk phage DNA in two *Taq* I restriction digest fragments. There are 9-bp inverted-repeat sequences beginning at positions 161 and 466 and the pivot point of rotation is at position 318. The positions of cleavage by the restriction enzymes *Hin*fI and *Tha* I are shown and are in excellent agreement with the ≈300 bp and ≈260 bp differences that were calculated from the on and off orientations observed with the λ412 phage and the *E. coli* genome Southern blots when the DNA preparations were digested with the respective restriction enzymes. The DNA sequence determined for the off orientation is also presented for the sections of DNA that include the upstream (Fig. 3*b*) and downstream (Fig. 3*c*) 9-bp inverted-repeat sequences. The sequence that is identical in both orientations is presented in uppercase letters and the sequences that are different due to the inversion are shown in lowercase letters.

The DNA sequence we have determined from the off orientation is in good agreement (although with minor differences) with that reported by Klemm (10) and by Orndorff and Falkow (11). Both of these published sequences are in the off switch orientation and neither contains a −10 or −35 pro-

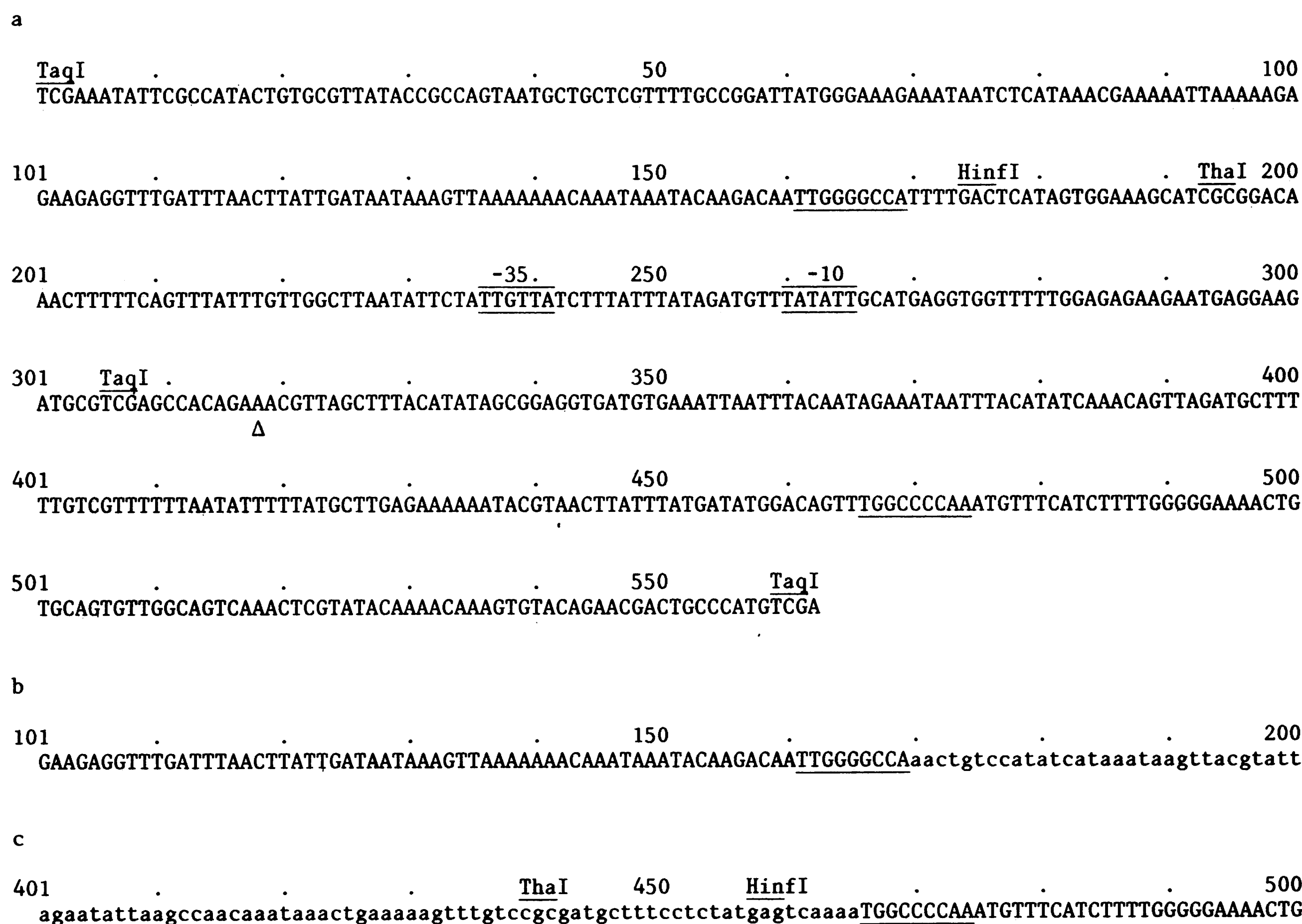

FIG. 3. The sequence of the DNA located upstream of the fimbrial structural gene. (*a*) This DNA sequence contains the invertible element and is that which is found in the positive, or on, orientation. The 9-bp inverted-repeat sequences are underlined and the positions of the *Hin*fI and *Tha* I restriction enzyme cleavage sites are shown. The −10 and −35 promoter regions are each overlined and underlined and are in the proper orientation for transcription of the structural gene, which is located ≈40 bp downstream from the *Taq* I site at position 559. The pivot point of inversion is designated by the open triangle below position 318. (*b* and *c*) The DNA sequences that include the upstream and downstream 9-bp inverted-repeats as found in the off orientation are shown in *b* and *c*, respectively. The DNA sequence that is identical to that found in the on orientation is shown in uppercase letters and the DNA sequence that is different as a result of the DNA inversion is shown in lowercase letters. The positioning of the *Hin*fI and *Tha* I restriction sites relative to the downstream inverted-repeat sequence as found in the off orientation is shown in *c*.

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moter region that is in very good agreement with *E. coli* promoter consensus sequences. When the DNA inverts to the on position, a near-consensus (5 of 6 bases) −10 region and a near-consensus (4 of 6 bases) −35 region are properly oriented with respect to the fimbrial structural gene, which begins ≈40 bp downstream from the *Taq* I site at position 559 (Fig. 3*a*). It is not possible to determine from this data whether the 9-bp inverted repeat sequences are actually part of the invertible element or are a recognition boundary of some type. For reasons of simplicity we have included these 18 bp in the overall size description of inversion of 314 bp, since these bases may actually be inverted during the phase-variation switching. This 9-bp sequence, 5′ TTGGGGCCA 3′, is unlike the consensus inverted-repeat sequence for the previously described class of prokaryotic invertible elements (12, 13).

DISCUSSION

Several prokaryotic systems have been described in which some type of surface determinant demonstrates phase variation. In *Neisseria gonorrhoeae*, a cell can alternately express several antigenically distinct pili, mediated by a cassette-type gene rearrangement in which silent gene copies of pilus antigens are moved into expression sites (14). In contrast, the well-studied, alternate expression of the H1 and H2 flagellar antigens of *Salmonella typhimurium* is due to the inversion of a 995-bp segment of DNA which is mediated by a site-specific recombinase enzyme that is the product of the *hin* gene (5, 12). A number of other invertible elements have been described that appear to belong to the same genetic class as that of *S. typhimurium*. Thus, the G-loop of Mu phage and the C-loop of P1 phage are each acted upon by phage-encoded recombinases (i.e, Gin and Cin, respectively) that are cross-complementable with Hin (13). Recently, a functionally related invertible segment of 1800 bp has been described for some *E. coli* K-12 strains (15), which is believed to be part of a defective prophage e14 (16). This element maps at 25 min on the chromosome, is controlled by a Hin-like protein, but gives rise to no obvious oscillating phenotype (16). It is clearly not involved in phase variation of type 1 fimbriae.

The on-and-off switch that drives *fimD* [now redesignated *fimA* (10)] maps near 98 min (17); here we have shown that it is mediated by an inversion of a DNA segment of 314 bp that is immediately upstream of the gene. This invertible element is unlike those previously described in that it does not encode its own recombinase and that it does not respond genetically to the *hin* gene product in *trans* (3). Moreover, the 9-bp inverted-repeat sequences bear no resemblance to the larger inverted-repeat sequences found in the previously described class of invertible elements. Thus we have identified an example of a different class of such elements, only the second such class to be found in prokaryotes. The DNA sequence of this element in the off orientation has been published by others (10, 11). The sequence contains a region that closely resembles the consensus −10 and −35 sites for *E. coli* transcription. It is in the proper orientation to act as a promoter in the on position but is oriented in the opposite direction and on the antisense strand in the off position.

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