Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 6506–6510, September 1987
Genetics

Integration host factor is required for the DNA inversion that controls phase variation in *Escherichia coli*

(site-specific recombination/gene expression/type 1 fimbriae/λ integrase/trans-active factors)

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Communicated by Martin Gellert, May 26, 1987 (received for review April 1, 1987)

ABSTRACT **The on-and-off expression (phase variation) of type 1 fimbriae, encoded by *fimA*, in *Escherichia coli* is controlled by the inversion of a promoter-containing 314-base-pair DNA element. This element is flanked on each side by a 9-base-pair inverted, repeat sequence and requires closely linked genes for inversion. Homology analysis of the products of these genes, *fimB* and *fimE*, reveals a strong similarity with the proposed DNA binding domain of λ integrase, which mediates site-specific recombination in the presence of integration host factor. Integration host factor, encoded by *himA* and *hip/himD*, binds to the sequence 5′ TNYAANNNRTTGAT 3′, where Y = pyrimidine and R = purine, in mediating integration–excision. In analyzing the DNA flanking the *fim* 314-base-pair inversion sequence, we found the adjacent sequence 5′ TTTAACTTATTGAT 3′, which corresponds perfectly with the consensus integration host factor binding site. To characterize the role of *himA* in phase variation, we transduced either a deletion of *himA* or an insertionally inactivated *hip/himD* gene into an *E. coli* strain with a *fimA–lacZ* operon fusion. We found the rate of phase variation decreases sharply from 10^{-3} to $<10^{-5}$ per cell per generation. Southern hybridization analysis demonstrates that the *himA* mutation results in a failure of the switch-generated genetic rearrangement. When the transductant was transformed with a *himA*$^+$ plasmid, normal switching returned. Thus integration host factor is required for normal type 1 fimbriae phase variation in *E. coli*.**

Genome rearrangement mediated by site-specific recombination is of widespread importance in the control of gene expression in prokaryotes and eukaryotes (1). The molecular basis for the mechanisms has been determined in several systems, including bacteriophage integration–excision (2, 3) and the interrelated invertible DNA elements best exemplified by the flagellar switch in *Salmonella* (4–6). In both systems the following three sets of factors are required. (*i*) The cis-specific DNA that acts as the crossing point for the rearrangement. (*ii*) Site-specific trans-active factors whose genes map near the cis-specific DNA. For example, in λ integration the *int* gene is carried by the bacteriophage (7–9), and in the *Salmonella* switch the *hin* gene is located within the invertible DNA (5). (*iii*) "Host factors" whose genes reside on the bacterial chromosome distant from the site of rearrangement. The best studied host factor is the integration host factor (IHF) of *Escherichia coli* required for λ phage integration (10). IHF consists of two subunits: IHFα, a M_r 10,500 polypeptide encoded by *himA* that maps at 38 min, and IHFβ, a M_r 9500 polypeptide encoded by *hip/himD* that maps at 20 min (11–14).

Like *Salmonella* flagellar expression, type 1 fimbriae of *E. coli* exhibit phase-variation control. We have shown that *E. coli* phase variation is transcriptionally regulated (15) and that the oscillating expression is due to the specific inversion of a 314-base-pair (bp) invertible element of DNA that directs transcription of *fimA* in one orientation of the switch only (16). Type 1 fimbriae mediate mannose-sensitive adherence of *E. coli* to eukaryotic cells including mucosal and phagocytic cells. The ability to turn on and off fimbriae expression at a high frequency (i.e., $\approx 10^{-3}$ switches per cell per generation) is likely to be important in permitting the bacteria to colonize in the early stage of infection (fimbriae "on" mode) and to avoid phagocytosis in the invasive stage of infection (fimbriae "off" mode).

Genetic studies indicate that, in addition to the 314-bp invertible element, trans-active factors mapping adjacent to the switch are required for the DNA inversion (17, 18). Based on the sequences of the cis-specific DNAs and the complementation analyses of the trans-active factors, the fimbriae switch and the *Salmonella* class of switches are biochemically different (16, 17).

We now present evidence that the fimbriae switch also requires another trans-active host factor for its inversion. Remarkably the host factor is the IHF needed for λ phage integration. Moreover, the fimbriae-specific trans-active factors, FimB and FimE, are significantly homologous to their λ phage counterpart, Int.

MATERIALS AND METHODS

Bacterial Strains and Media. *E. coli* strain VL386 is a derivative of K-12 strain CSH50 with a *lac* operon fusion in the fimbriae structural gene (19). Because strain CSH50 has a deletion of the *lacZ* gene, expression of *lac* in strain VL386 undergoes phase variation (Lac$^+$ ⇌ Lac$^-$) controlled by inversion of the element containing the Fim promoter. The IHF mutant strains were constructed by P1 transduction of VL386 (20). The donor *E. coli* strains were K1299 (21), which carries the *himAΔ82* mutation with an associated Tn*10* (22), and K2704, containing an insertion in the *hip/himD* gene of the gene encoding chloramphenicol resistance (Cm^r) from pBR325 (23). Because both names have been used for the latter IHF gene, we have chosen the indicated nomenclature to avoid confusion. Strains VL801 and VL802, the resulting *himA* transductants, were then transformed with phimA-4, a pBR322 plasmid carrying a functional *himA* gene to yield strains VL803 and VL804, or with phimA-4Δsma to yield strains VL805 and VL806. Plasmid phimA-4 was kindly supplied by H. Miller (Genentech) (22). The phimA-4Δsma plasmid, constructed by A. Granston (University of Michigan, Ann Arbor, MI), is a derivative of phimA-4 with an internal deletion of the *himA* gene. Strains VL807 and VL808, the *hip/himD* transductants, were transformed with pE313, a pBR322 plasmid carrying a functional *hip/himD* gene, to yield strains VL809 and VL810. Plasmid pE313 was kindly supplied by E. Flamm and R. Weisberg (National Institutes of Health, Bethesda, MD) (23).

Phase variation rates of the Fim phenotype were determined on MacConkey and 2,3,5-triphenyltetrazolium chlo-

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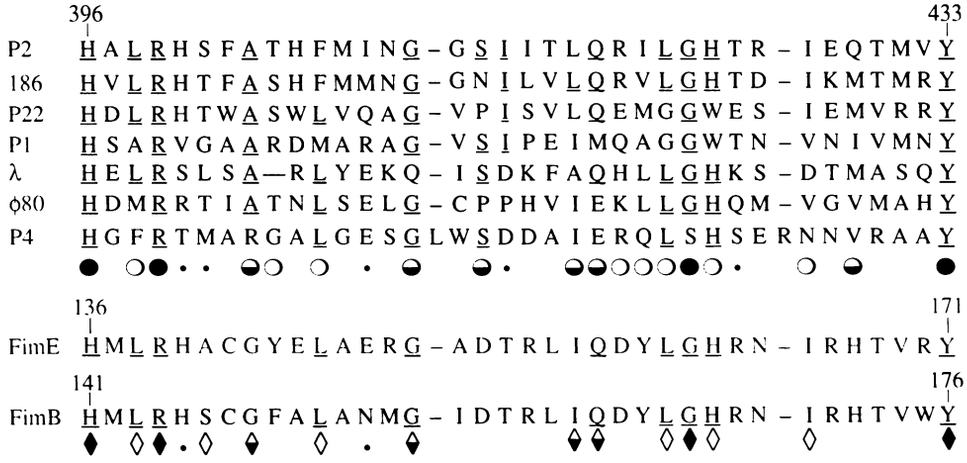

FIG. 1. C-terminal alignment of the phage integrase proteins and the FimB and FimE proteins of *E. coli*. (*Upper*) Comparative analysis of the integrase proteins performed by Argos *et al.* (28). Same-residue conservation in >50% of the proteins aligned in that position is indicated by underlining the conserved amino acids. Substitutions were defined as conservative if both amino acids fall into one of the following exchange groups as defined by Dayhoff *et al.* (29): serine, proline, alanine, glycine, and threonine; arginine, lysine, and histidine; phenylalanine, tryptophan, and tyrosine; aspartic acid, glutamic acid, glutamine, and asparagine; isoleucine, leucine, methionine, and valine; cysteine. Solid circles are below positions for which all seven aligned amino acids are in the same exchange group. Half-open and open circles are below positions for which six and five amino acids are in the same exchange group, respectively. Dots are below positions for which four amino acids are in the same exchange group and at least three of the four are identical. We performed a similar analysis of the FimB and FimE proteins. (*Lower*) Comparison of amino acids 141 to 176 of the FimB protein and amino acids 136 to 171 of the FimE protein to the phage proteins. The FimB and FimE residues that are also found in at least four of the integrase proteins are underlined. Solid diamonds are below positions for which all nine aligned amino acids are in the same exchange group. Half-open and open diamonds are below positions for which the FimB and FimE residues are in the same exchange group as six or five of the integrase residues, respectively. Dots are below positions for which six amino acids are in the same exchange group and at least four of the six are identical.

ride indicator plates, as described (15). All routine media were prepared as described by Miller (24).

DNA Preparations. DNA isolation and high stringency Southern hybridization analysis were performed as described by Maniatis *et al.* (25). A 715-bp *Ava* I–*Hpa* I fragment of pJLA2 (16) containing the 314-bp inversion sequence was radiolabeled with [α-^{32}P]CTP (Amersham) using the "oligo-labeling" technique of Feinberg and Vogelstein (26) and used to probe chromosomal DNA digested with *Hin*fI. Restriction enzyme digestions were conducted as suggested by the vendors (New England Biolabs and Bethesda Research Laboratories).

RESULTS

Homology Between λ Integrase and the FimB and FimE Proteins. We have shown that phase variation in *E. coli* requires both a cis-active switch (15, 16) and a closely linked trans-active factor (17). Klemm (18) has cloned two genes, *fimB* and *fimE*, that are adjacent to the invertible switch and are required in trans for switch inversion. Analysis of the DNA sequences of these genes has shown that their predicted products are highly homologous (18).

We compared the predicted amino acid sequences of FimB and FimE to the 2677 protein sequences in the National Biomedical Research Foundation* library using the programs described by Lipman and Pearson (27). The λ integrase protein was identified as one of the most homologous proteins to FimB, having a score of 87 (representing 7.33 standard deviations above the mean) when their sequences are optimally aligned. Some homology (i.e., >2 standard deviations above the mean) with FimB was also noted for the integrase proteins of bacteriophages P2, 186, P22, and P1, and some homology between FimE and integrase proteins of bacteriophages 186 and φ80 was found. No homology was seen with Xis, the λ-encoded protein required for prophage excision from the chromosome. Argos *et al.* (28) have shown that this group of integrase proteins shows greatest homology in a proposed DNA-binding region of ≈40 amino acids (Fig. 1), which is the region of maximal homology with the Fim proteins. Moreover, this region of homology in the C terminus of the Fim proteins is their region of maximal homology with each other (Fig. 1). FimB and FimE, like these site-specific recombinases, are basic proteins (estimated pI value is 12).

The Role of IHF in Fimbrial Phase Variation. The transcriptional switch controlling the phase variation of type 1 fimbriae is the 314-bp invertible segment of DNA that is situated just upstream of the fimbriae structural gene *fimA*. By analogy with other well-characterized site-specific recombination systems, it might be expected that, in addition to the genetically linked trans-active factors, there would exist nonlinked host factors required for recombination. Since we found that FimB and FimE, the genetically linked trans-active factors for fimbriae switch inversion, share homology with λ integrase, the linked factor for λ integration, we next determined whether the fimbriae DNA switch was dependent on IHF.

To determine if IHF plays a role in phase variation, we measured the rate of inversion in a host deficient in IHF activity. Two genetic modifications were employed in these studies. First, for ease of measuring Fim variation, we employed a strain of *E. coli* that contains a *fimA–lacZ* operon fusion. Because this strain is deleted in the normal *lacZ* gene, expression of Lac undergoes phase variation ($Lac^+ \rightleftharpoons Lac^-$) controlled by inversion of the element containing the Fim promoter. Second, we transferred a deletion mutation of *himA* by P1 transduction. This mutation renders the cell defective for the α subunit of IHF.

Table 1 shows the results of the experiments. When the wild-type *himA* gene is present, normal phase variation is observed—i.e., there is a switch at the expected frequency from Lac^+ to Lac^- or from Lac^- to Lac^+ depending on the state of the starting strain. When bacteria with the fusion

*Protein Identification Resource (1985) Protein Sequence Database (Natl. Biomed. Res. Found., Washington, DC), Release 6.0.

Table 1. Phase variation rates of IHF$^-$ and IHF$^+$ derivatives of operon fusion strain VL386

Strain	Genotype	Phenotypic state	Phase variation rate
VL386	*fimA-lacZ*	Lac$^+$	$8.6 \pm 4.0 \times 10^{-4}$
		Lac$^-$	$3.2 \pm 2.8 \times 10^{-3}$
VL801	VL386 *himAΔ82*	Lac$^+$	5×10^{-6}
VL802	VL386 *himAΔ82*	Lac$^-$	5×10^{-6}
VL803	VL801 phimA-4	Lac$^+$	$4.2 \pm 0.9 \times 10^{-3}$
		Lac$^-$	$3.2 \pm 3.2 \times 10^{-3}$
VL804	VL802 phimA-4	Lac$^+$	$2.5 \pm 4.0 \times 10^{-3}$
		Lac$^-$	$4.4 \pm 4.5 \times 10^{-3}$
VL805	VL801 phimA-4Δsma	Lac$^+$	$<1 \times 10^{-5}$
VL806	VL802 phimA-4Δsma	Lac$^-$	$<3 \times 10^{-5}$
VL807	VL386 *hip/himD*	Lac$^+$	2×10^{-5}
VL808	VL386 *hip/himD*	Lac$^-$	$<3 \times 10^{-6}$
VL809	VL807 pE313	Lac$^+$	$4.7 \pm 2.6 \times 10^{-3}$
		Lac$^-$	$3.6 \pm 1.2 \times 10^{-3}$
VL810	VL808 pE313	Lac$^+$	$2.6 \pm 2.3 \times 10^{-3}$
		Lac$^-$	$4.1 \pm 3.7 \times 10^{-3}$

Phase variation rates from Lac$^+$ to Lac$^-$ and Lac$^-$ to Lac$^+$ were calculated by the formula $(M/N)/g$ where M/N is either the ratio of Lac$^-$ cells to total cells or Lac$^+$ cells to total cells, and where g is the number of generations of growth from a single cell to the number of cells within a colony as described (15). In all cases, cells that had undergone phase variation were tested for their ability to switch back to the original Lac phenotype and were found to do so at about the same frequency.

carry a *himAΔ82* mutation, markedly diminished switching is observed whether the starting strain is in the "on" (Lac$^+$) or "off" (Lac$^-$) configuration.

To prove that the failure in normal switching is due to the presence of the *himA* mutation, we tested whether the low-switching phenotype could be reversed by complementation with a wild-type *himA* allele. A multicopy plasmid carrying the *himA*$^+$ allele, phimA-4, was transformed into both Lac$^+$ and Lac$^-$ *himAΔ82* low-switching derivatives with the *fimA–lacZ* fusion. As shown in Table 1, the switching defect was reversed in the presence of the plasmid. To prove that the complementation specifically resulted from the expression of *himA*, we tested whether a derivative of phimA-4 that carries an internal deletion of the *himA* gene, phimA-4Δsma, could similarly complement for switching. When phimA-4Δsma was transferred to the low switching *himA* strains, there was no correction of the defect in phase variation. Therefore, we conclude that IHFα, encoded by the *himA* gene, is required for the phase variation that alters the expression of type 1 fimbriae.

Since IHF can influence gene expression by mechanisms other than recombination (30–35), it was necessary to determine if the *himA* mutation results in a failure of the switch recombination *per se* or if it influences *fimA* expression in some other manner. To distinguish between these possibilities, we analyzed by Southern hybridization the orientation of the invertible element in the various *him* derivatives.

Because sites for several different restriction enzymes are asymmetrically located within the invertible element, digestion of chromosomal DNA with one of these enzymes (e.g., *Hin*fI) will give different sized fragments for this region depending on the orientation of the switch. By employing a probe that includes the invertible DNA, we were able to identify these phase-characteristic restriction fragments (Fig. 2). Analysis of the wild-type DNA isolated from a single colony grown to 10^9 cells gives physical evidence of the normal switching frequency. These studies reveal a hybridization pattern marked by a predominance of DNA in one orientation with a minority of DNA, seen as secondary bands, that derives from 2 to 5% of cells in which the switch is in the opposite orientation. In the case of bacteria with the *himAΔ82* mutation, no secondary bands are observed. Thus, there is no physical evidence of recombination of the invertible switch under physiological conditions where there is no active IHFα. This observation demonstrates that the *himA* gene product is involved in the actual recombination event required for switching.

In other experiments, we have found similar frequency-of-switching results to those of *himA* with a mutation in the *hip/himD* gene (Table 1). Thus, as in other reactions requiring IHF, both α and β subunits are required for the site-specific recombination involved in fimbrial phase variation.

The Region Adjacent to the Invertible Region Contains a Consensus IHF Binding Site. The consensus sequence for IHF

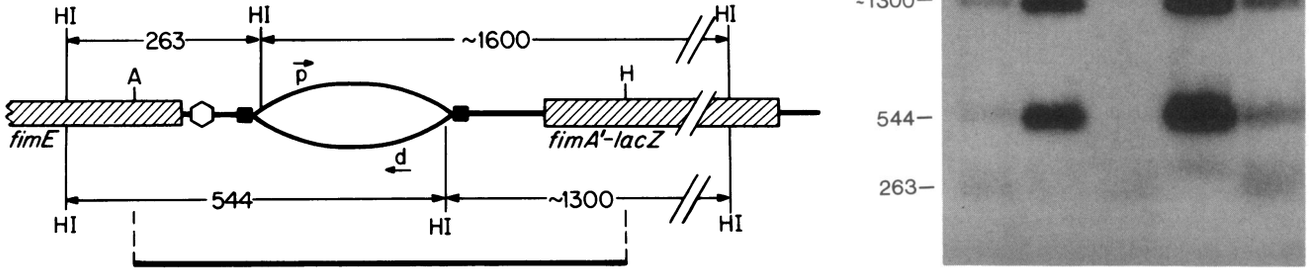

FIG. 2. (*Left*) Schematic representation of the invertible DNA element controlling phase variation of fimbriae, including the position of the promoter (p), the IHF binding site (○), and the inverted repeats (■). The relative locations of *fimA* and *fimE* are noted; *fimB* terminates 478 bp upstream from *fimE* (18). The direction of transcription of *fimE* is left-to-right, relative to the orientation of *fimE* in the figure (18). Restriction sites indicated are the chromosomal *Hin*fI (HI) sites and the *Ava* I (A) and *Hpa* I (H) sites used to construct the Southern probe, shown as the thick line with dashed boundaries. Shown for both orientations of the switch are the predicted hybridizing band sizes in the "on" (above the element) and "off" (below the element) configurations. (*Right*) Southern hybridization analysis of chromosomal DNAs digested with *Hin*fI and probed with the 715-bp *Ava* I–*Hpa* I fragment from pJLA2. Shown are DNAs from strain VL386 in Lac$^+$ phase (lane a), strain VL386 in Lac$^-$ phase (lane b), the *himA* derivative of Lac$^+$ VL386 (lane c), the *himA* derivative of Lac$^-$ VL386 (lane d), and the *himA* derivative of Lac$^+$ VL386 containing phimA-4 (lane e). Size markers are in base pairs.

binding is 5′ TNYAANNNRTTGAT 3′, where Y = pyrimidine and R = purine (36). In analyzing the DNA sequence of the switch region we found one sequence perfectly homologous with the consensus IHF binding site. Based on our published switch sequence (16), the IHF site is found at residues 113–126 (5′ TTTAACTTATTGAT 3′), whereas the left 9-bp inverted repeat is found at residues 161–169 (Fig. 2). Thus, the consensus IHF binding site residues are close to but outside of the 314-bp segment of invertible DNA.

DISCUSSION

The experiments presented in this paper demonstrate that inversion of the genetic switch controlling expression of the *E. coli fimA* gene requires the participation of IHF. IHF, which is composed of two subunits, was first identified by Nash and coworker (10) because of its role in the site-specific recombination reactions that catalyze the integration and excision of the λ coliphage genome into and out of the bacterial chromosome. Subsequent genetic studies identified two genes, *himA* and *hip/himD*, that encode the α and β subunits of IHF, respectively (11, 12, 14). The IHF dimer is a member of the class II bacterial DNA-binding proteins (22). This class of proteins has been recognized as the bacterial analogue of histone proteins (37).

Studies with the *himA* and *hip/himD* mutants have identified a number of physiological roles for IHF, primarily influencing the expression of both bacterial and phage genes (30–35). Other than the initial observation of IHF involvement in λ site-specific recombination as well as the demonstration that IHF is required for the integration of other lambdoid phages, to our knowledge, no study has implicated a role for IHF in a recombination reaction. It has been reported that sequences essential for transposition of IS*1* contain a consensus IHF binding sequence (38). However, we know of no evidence that IHF is required for this transposition. Thus, our finding of a requirement for IHF in the normal recombination reaction controlling *fimA* expression offers not only a second example of IHF involvement in recombination, but the first reported example of a host-specific recombination reaction requiring IHF. We expect that additional roles for IHF in recombinational events will be discovered—e.g., unreported studies implicate IHF in the transposition of Tn*10* (D. Morisato and N. Kleckner, personal communication). Although we have identified a sequence that matches perfectly with the consensus IHF binding site reported by Craig and Nash (36), we, as yet, have no evidence as to whether that site binds IHF.

Other examples of regulation of gene expression by an invertible genetic element are well documented. The best-studied case is the expression of the *Salmonella* flagellin genes. Interestingly, IHF has been shown to play a role, not in the recombination reaction inverting the control element, but directly in controlling the expression of the flagellin gene product (31). Because the recombinase protein and DNA sites involved in the flagellar switch system show homologies with analogous systems in transposons, in phages Mu and P1, and in the cryptic prophage e14, it has been proposed that these elements evolved from a common ancestor (39).

In the case of the switch controlling *fimA* expression, we have demonstrated that the products of the *himA* and the *hip/himD* genes are involved in the recombination reaction that inverts the genetic element and its internal, unidirectional promoter. The Southern hybridization experiment shows that the inversion of the DNA element in a *himA* deletion mutant is beneath detectable levels regardless of whether the strain is in the "on" or "off" configuration. Based on the location of an IHF binding site near the switch (Fig. 2), we presume that the IHF effect is direct. Nevertheless, it is conceivable that the IHF effect may be mediated by an indirect process (i.e., regulation of expression of another product).

The fact that IHF participates in the site-specific recombination of lambdoid phages and in the inversion of the *fimA* switch is consistent with the results of the computer search that revealed significant homologies between the *fimB* and *fimE* gene products required for switching and the λ *int* gene product. These results suggest a common evolutionary ancestry for the components of the recombination reactions involved in site-specific recombination and fimbrial phase variation, paralleling the proposed common evolutionary heritage of the flagellar class of switches and transposons. Whether the phage borrowed or contributed the recombination system is not apparent. If they do share a common evolutionary past, phage site-specific recombination and fimbrial phase variation have diverged in at least one striking way; the former reaction is highly efficient, while the latter is relatively inefficient.

The relatively lower frequency of normal fimbrial phase variation may be important for the growth of *E. coli* in one of its natural environments, the human host. According to this idea, the oscillation between the two states is necessary to allow first for the colonization of mucosal surfaces by fimbriated bacteria, and second for avoidance of phagocytosis (by nonfimbriated bacteria) once invasion past the superficial mucosal layer has occurred. Presumably, the rate of phase variation has been selected as the optimal one for the survival of the infecting population of bacteria.

We thank Andrew Granston and Al Schauer for helpful discussions. This work is supported in part by Public Health Service Research Grants AI14363 and AI24734 and by a Research Career Development Award (B.I.E.) from the National Institutes of Health.

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