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Genetic Analysis of the Phase Variation Control of Expression of Type 1 Fimbriae in *Escherichia coli*

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Expression of type 1 fimbriae in *Escherichia coli* exhibits phase variation, whereby individual cells can alternate between states of organelle expression (Fim$^+$) and nonexpression (Fim$^-$). Strains with a *fimD-lac* operon fusion, in which *lac*, rather than *fimD*, expression is under the control of the *fimD* promoter, undergo Lac$^+$ ⇌ Lac$^-$ phase variation, instead. After positioning a λ prophage adjacent to the operon fusion, we were able to isolate specialized λ phage carrying both the *fimD-lac* fusion and the phase variation control region. Introduction of such phage into an Fim$^+$ strain resulted in construction of a strain with a double, independently switching phenotype (Fim$^+$ ⇌ Fim$^-$ and Lac$^+$ ⇌ Lac$^-$), demonstrating that the region controlling phase variation is contiguous with the *fimD-lac* operon fusion and is *cis* acting. When the specialized λ phage was propagated on a Δ*lac* Δ*fim* strain, phase variation occurred within the plaques, confirming that the phase variation control region is carried on the specialized transducing phage. All lysogens acquired the Lac$^+$ ⇌ Lac$^-$ phenotype, except for two nonswitching Lac$^+$ recombinants, which acquired Lac$^+$ ⇋ Lac$^-$ phase variation only by *trans* complementation with *fim*. Phase variation of type 1 fimbriae, therefore, appears to involve both a *cis*-active element, which is cloned on a specialized λ phage, and a *trans*-active permissive factor, which is not present on the phage, but rather must be supplied by the recipient strain in the transduction.

Phase variation has been described for many procaryotic systems (9, 14, 15, 21, 28) and is perhaps best characterized genetically for alternate expression of two distinct flagellar antigens, H1 and H2, in *Salmonella typhimurium* (28). In *Escherichia coli* oscillation of type 1 fimbriae between on and off states of expression is also regulated by phase variation-type control (9), but the molecular mechanisms of this phase variation have not yet been explored in detail. Type 1 fimbriae, which are found on the majority of clinical isolates of *E. coli* (8), are proteinaceous appendages that mediate mannose-sensitive binding of bacteria to a variety of eucaryotic cells (2, 27). The ability to switch between fimbriation and nonfimbriation may be an important virulence factor, since the presence of fimbriae may be advantageous or detrimental to the organism, depending on whether the organism is attaching to epithelial cells or to leukocytes (10).

Using the in vivo operon fusion method (6), we have previously shown that the switch is controlled at the level of transcription and that the oscillation between states of fimbriation and nonfimbriation occurs randomly at a frequency of approximately one switch per 1,000 cells per generation (9). Although the exact mechanism of the switch is unknown at present, phase variation of fimbrial expression would appear to be a genetic event, similar to that of flagellar phase variation in *S. typhimurium* (28) or pilus phase variation in *Neisseria gonorrhoeae* (21).

To study the genetics of the system in more detail, we positioned a λ prophage adjacent to the original *fimD-lac* operon fusion (13); as expected, this strain is Fim$^-$, but capable of Lac$^+$ ⇌ Lac$^-$ phase variation. In this paper, we describe experiments based on the use of specialized λ phage isolated from this lysogen. We have found that this phage carries the *cis*-active switch as well as the *fimD-lac* operon fusion, and that the switch is expressed both during lytic infection and during lysogenization. We have also identified a *trans*-active factor, encoded by the bacterial genome, but not the specialized phage, that is required for oscillation of the *cis*-active switch and expression of the phase variation phenotype.

MATERIALS AND METHODS

Chemicals and media. Tetracycline hydrochloride, streptomycin, spectinomycin, 2,3,5-triphenyl tetrazolium chloride, ampicillin, acridine orange, and 5-bromo-4-chloro-3-indolyl-β-D-galactoside were obtained from Sigma Chemical Co. (St. Louis, Mo.). Tryptone, yeast extract, and MacConkey agar were from Difco Laboratories (Detroit, Mich.). All other chemicals were of reagent grade and were readily available.

We used LB medium (22) for routine bacterial culturing and modified λYM medium (7) for growing cells for λ infection. λYM medium contains 10 g of tryptone, 2.5 g of NaCl, and 0.1 g of yeast extract per liter of distilled water supplemented with 0.2% maltose and 20 mM $MgSO_4$. Tetracycline, where required, was added at a concentration of 7.5 μg/ml in MacConkey or 2,3,5-triphenyl tetrazolium chloride indicator plates and at 15 μg/ml elsewhere. Streptomycin and spectinomycin, where required, were each added at a concentration of 100 μg/ml. Amino acids, where required, were added at a concentration of 20 μg/ml.

2,3,5-Triphenyl tetrazolium chloride lactose indicator agar and 5-bromo-4-chloro-3-indolyl-β-D-galactoside indicator agar were prepared as described by Miller (22). Fusaric acid agar was prepared as described by Bochner et al. (3), as modified by Maloy and Nunn (18).

Bacterial and phage strains. The *E. coli* strains used in this study are listed in Table 1. Phage strain λNK55 (16) was obtained from C. Rock, λ *cI b2* was from J. Miller (22), λ *cI h80* was from T. Silhavy, and P1 *vir* was from P. Bassford.

Preparation of λ phage lysates. UV irradiation of bacterial

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TABLE 1. *E. coli* strains[a]

Strain	Genotype or phenotype	Source, reference or construction
CSH50	*ara* Δ(*lac-pro*) *rpsL thi*	J. Miller (22)
CGSC4250	*thr-1 leuB6 proA2 his-4 recA13 argE3 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 crpsL31 tsx-33 supE44* λ⁻ F′ *fim*⁺ *thr*⁺ *leu*⁺	B. Bachmann
S10	*lacZ relA lysA* Δ*cya854 ilv*::Tn*5 argE*::Tn*10 rpsE*	P. Bassford
MCL30	HfrPO45 *thi-1* Δ(*srl-recA*)*306*::Tn*10*	M. C. Lorence (17)
MCL31	HfrPO201 Δ(*gpt-lac*)*5 relA1 rpsE2123 thi-1 supE44* TP3 Δ(*srl-recA*)*306*::Tn*10*	M. C. Lorence (17)
HB101(pJZ110)	HB101, pJZ110 Hin⁺	M. Siverman (32)
VL361	CSH50 ϕ(*fimD-lac*) Mu *dI*[b]	This laboratory (9)
VL386	VL361, λp1(209), Δ(Mu *dI*)[c]	This laboratory (13)
VL412	CSH50, λ386	Lysogenization of CSH50 with λ386 (phage lysate isolated from UV induction of VL386)
VL561	CGSC4250 (cured of F′ factor)	Treatment with acridine orange (5 μg/ml)
VL564	VL561 Spc[r]	Spontaneously resistant to spectinomycin (100 μg/ml)
VL565	CSH50 *rpsE rpsL*⁺	Transduction of CSH50 to Spc[r] Str[s] (donor S10)
VL620 through VL623	VL564 F′ Tn*10 fim*⁺	Conjugation of VL564 with Tn*10*-mutagenized CGSC4250, selection for Tet[r] Spc[r]
VL648	CSH50, λ412, Lac⁺ ⇌ Lac⁻	Lysogenization of CSH50 with λ412 (phage lysate isolated from UV induction of VL412)
VL649	VL584, λ412, Lac⁺ ⇌ Lac⁻	Lysogenization of VL584 with λ412 (phage lysate isoalted from UV induction of VL412)
VL664	VL584, λ412, Lac⁺ (phase locked)	Lysogenization of VL584 with λ412 (phage lysate isolated from UV induction of VL412)
VL650 through VL657	VL386, F′ Tn*10 fim*⁺	Conjugation of VL386 with VL620, selection for Tet[r]
VL667 through VL671	VL565 F′ *fimD-lac*	Conjugation of VL565 with VL650, selection for Spc[r] Tet[r]
VL674	VL386 Δ(*srl-recA*)*306*::Tn*10*	Conjugation of VL386 with MCL31, selection for Tet[r] Str[r]
VL676	VL664 Δ(*srl-recA*)*306*::Tn*10*	Conjugation of VL664 with MCL31, selection for Tet[r] Str[r]
VL707	VL674 Δ(Tn*10*)	Tet[s] derivative of VL674, selection on fusaric acid plates
VL709	VL676 Δ(Tn*10*)	Tet[s] derivative of VL676, selection on fusaric acid plates
VL734	VL565 Δ(*srl-recA*)*306*::Tn*10*	Conjugation of VL565 with MCL30, selection for Tet[r] Spc[r]
VL738	VL709 F′ Tn*10* fim⁺	Conjugation of VL709 with VL620, selection on minimal glucose-thiamine-proline tetracycline plates
VL739 through VL741	VL738 (cured of F′ Tn*10* fim⁺), Lac⁻ (phase locked)	Treatment with acridine orange 12.5 μg/ml
VL743	VL734 Δ(Tn*10*)	Tet[s] derivative of VL734, selection on fusaric acid plates
VL745	*V*L743 F′ Tn*10 fim*⁺	Conjugation of VL743 with VL620, selectin on minimal glucose-thiamine-proline-tetracycline plates
VL747	VL664 F′ Tn*10 fim*⁺	Conjugation of VL664 with VL745, selection for Tet[r] Str[r]
VL749	VL747 (cured of F′ Tn*10* fim⁺), Lac⁻ (phase locked)	Treatment with acridine orange (12.5 μg/ml)
VL751	VL664 Δ(λ412)	UV induction of VL664, isolation of a Lac⁻ λ[s] strain
VL752	CSH50, λ412	Lysogenization of CSH50 with λ412 (phage lysate isolated from UV induction of VL412)
VL768	VL707 F′ Tn*10 fim*⁺	Conjugation of VL707 with VL745, selection for Tet[r] Str[r]
VL773	VL743 F′ Tn*10 fimD-lac*	Conjugation of VL743 with VL651, selection for Tet[r] Spc[r]

[a] Genetic nomenclature is that of Bachmann (1) and Campbell et al (5).
[b] The designation ϕ(*fimD-lac*) indicates that this strain has the *lac* genes inserted into, and transcribed with, the *fimD* as a result of lysogeny by the Mu *dI* bacteriophage. This strain alternatively can be shown as *fimD*::Mu *dI*.
[c] The designation λp1(209) indicates that this strain has been lysogenized with bacteriophage λp1(209) and the designation Δ(Mu *dI*) indicates that intervening Mu DNA sequences have been deleted.

strains carrying λ prophage was performed as described by Miller (22), with minor modifications. Single colony isolates of strains to be induced were grown in 10 ml of LB medium to cell densities of 2×10^8 to 3×10^8 cells per ml. Cells were harvested by centrifugation at 5,000 rpm for 10 min, suspended in 5 ml of 0.1 M $MgSO_4$, and irradiated with UV light for 40 s (an exposure time that allowed 50% cell survival). The irradiated cells were diluted with 5 ml of λYM medium, incubated at 37°C with aeration for 2.5 h, treated with chloroform, and centrifuged at 10,000 rpm for 10 min. Dilutions of the phage lysates (supernatants) were mixed with CSH50 cells grown in λYM medium and plated on 5-bromo-4-chloro-3-indolyl-β-D-galactoside indicator agar with F-top agar (22).

Construction of *fimD-lac* λ lysogens. Samples of strain CSH50 (Fim⁺) or strain VL584 (Fim⁻) grown in λYM

medium were mixed with various dilutions of λ phage lysates obtained from UV induction of different prophage-carrying strains. After a 10-min incubation to allow for λ adsorption, the cells were pelleted and suspended in 1.0 ml of LB medium. After 1 h of incubation at 37°C, cells were again pelleted and suspended in 100 μl of LB medium. The suspended cells were spread on MacConkey lactose agar with a mixture of λ *cI b2* and λ *cI h80* to select for λ lysogens. Single colony lysogens that were Lac^+ were restreaked onto MacConkey lactose agar for observation of $Lac^+ \rightleftharpoons Lac^-$ phase variation.

Construction of merodiploid strains via F′ transfer. To facilitate transfer of a F′ factor carrying *fim*$^+$ genes into a variety of recipient strains, we first constructed a derivative of strain CGSC4250 that carries a Tn*10* insertion in the F′ factor. CGSC4250 was mutagenized with λNK55 by the method of Kleckner et al. (16). A few thousand Tet^r cells were pooled with the expectation that some would have Tn*10* positioned on the F′ factor. The pooled Tet^r cells were used as the donor with strain VL564 (a spectinomycin-resistant, cured derivative of CGSC4250) serving as the recipient. The matings were carried out as described by Miller (22). Exconjugants were selected as resistant to spectinomycin and resistant to tetracycline. Several exconjugants that were also threonine and leucine prototrophs (the F′ carries *thr*$^+$, *leu*$^+$, and *fim*$^+$ genes) were saved (strains VL620-VL623).

One of these exconjugants (strain VL620) was used as the donor in a mating with strain VL386 (*fimD-lac* λ) as the recipient. Exconjugants were selected on MacConkey lactose agar containing tetracycline (lac-tet agar) to select against recipient cells. We did not directly select against the donor cells; however, control plates spread with diluted donor cells alone gave no growth after 18 h of incubation. (Donor colonies grew much slower than exconjugant colonies because of a *recA* mutation in the donor.) Lac^+ and Lac^- colonies were restreaked on lac-tet agar for observation of $Lac^+ \rightleftharpoons Lac^-$ phase variation. VL650 through VL657 are derivative strains of VL386 (*fimD-lac* λ) carrying the F′ factor from strain VL620.

For some of our experiments it was preferable to use strains carrying *recA* mutations to decrease the possibility of recombination between genes located on the F′ factor and genes located on the chromosome. We constructed *recA* derivative strains by mating either strain MCL30 [HfrPO45 Δ(*srl-recA*)*306*::Tn*10* Str^s Spc^s] or strain MCL31 [HfrPO201 Δ(*srl-recA*)*306*::Tn*10* Str^s Spc^r] with either Spc^r or Str^r derivatives of CSH50. Matings were done as described previously (17), and exconjugants were selected on plates containing tetracycline and either spectinomycin or streptomycin. Strains were then tested for enhanced UV sensitivity to demonstrate the presence of a *recA* mutation. Since it was necessary to have Tet^s *recA* derivative strains for transfer of F′ Tn*10 fim*$^+$, the *recA* derivative strains were plated on fusaric acid agar (3, 18). Tet^s derivative strains were then rechecked for UV sensitivity.

To construct strains carrying *fim*$^+$ chromosomal genes and the *fimD-lac* operon fusion on the F′ factor, a mating was performed with strain VL651 (*fimD-lac* F′ Tn*10 fim*$^+$) as the donor and strain VL743 (an Spc^r Tet^s *recA* derivative of CSH50) as the recipient. Since strain VL651 is *rec*$^+$, recombination can occur between genes on the chromosome and genes on the F′ factor. By selecting for Spc^r Tet^r exconjugants and screening for an Lac^+ phenotype on lac-tet agar with spectinomycin (lac-tet-spc agar), it is possible to detect transfer of an F′ factor that carries the *fimD-lac* fusion. Lac^+ colonies were restreaked onto lac-tet-spc agar for observation of $Lac^+ \rightleftharpoons Lac^-$ phase variation. Strain VL773 is one *recA* derivative strain of CSH50 (*fim*$^+$) carrying the *fimD-lac* operon fusion on the F′ factor.

Strain VL707 (a Tet^s Str^r *recA* derivative of VL386) was mated with strain VL745 (an Spc^r Str^s Tet^r derivative of CSH50 carrying F′ Tn*10 fim*$^+$). Exconjugants were selected on lac-tet-spc agar and restreaked on lac-tet-spc agar for observation of $Lac^+ \rightleftharpoons Lac^-$ phase variation. Strain VL768 is one derivative strain of VL707 (*fimD-lac*) carrying the F′ Tn*10 fim*$^+$.

Yeast cell agglutination. To determine the Fim^+ or Fim^- phenotype of bacterial strains, yeast cell agglutination was employed with *Candida* sp. cells as described previously (11).

Filter immune assay for detection of fimbria expression. Overnight cultures of CSH50, VL386, and VL752 were diluted and spread onto MacConkey lactose plates. Plates were incubated at 37°C for approximately 15 to 18 h. Bacterial colonies were directly transferred to dry nitrocellulose filters (Millipore Corp., Bedford, Mass.; HA, 0.45 μm). Filter immune assays were performed by the procedure of Meyer et al. (21), with minor modifications. Filters were placed on Whatman 3MM paper soaked in solutions of 0.5 N NaOH–1.5 M Tris-hydrochloride (pH 7.5)–2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium nitrate)–70% ethanol, stepwise for 5 min each and then dried and baked under vacuum at 60°C for 2 h. Filters were incubated with shaking in a solution of TTBS (20 mM Tris, 500 mM NaCl [pH 7.5], 0.05% Tween 20) for 1 h, followed by an overnight incubation in diluted anti-fimbria monoclonal antibody (diluted 1:100 in TTBS) (12). The following day the filters were washed with three changes of TTBS and then incubated for 2 h with shaking in horseradish peroxidase goat anti-mouse antibody (diluted 1:2,000 in TTBS). The washes were repeated with three changes in TTBS. The color development solution was prepared by dissolving 60 mg of Bio-Rad color development reagent into 20 ml of ice-cold methanol and then mixing with 600 μl of ice-cold 3% hydrogen peroxidase in 100 ml (total volume) of 20 mM Tris–500 mM NaCl (pH 7.5). Color development solution was poured onto filters after the third wash. Color development was stopped after 10 to 15 min by washing filters in water.

Transfer of F′ Tn*10 fim*$^+$ into phase-locked strain VL709. An *recA* derivative of strain VL664 was constructed by mating with strain MCL30 [HfrPO45 Δ(*srl-recA*)*306*::Tn*10* Str^s Spc^s]. Exconjugates were selected on agar containing tetracycline and streptomycin. Matings were done as described previously (18), and exconjugants were selected on agar containing tetracycline and either spectinomycin or streptomycin. Strains were then tested for enhanced UV sensitivity to demonstrate the presence of an *recA* mutation. Since it was necessary to have Tet^s *recA* derivative strains for transfer of F′ Tn*10 fim*$^+$, the *recA* derivative strains were plated on fusaric acid agar (3, 18) and then tested for tetracycline sensitivity. These strains were then rechecked for UV sensitivity; one of the strains (VL709) was then used in further experiments.

Strain VL709 was then mated with VL620 (F′ Tn*10 fim*$^+$), and Str^r Tet^r exconjugates were selected. These were then streaked onto MacConkey lactose agar to determine whether Lac phase variation was occurring. Several exconjugates were then cured of the F′ factor by using acridine orange at a final concentration of 12.5 μg/ml as described by Miller (22).

Transformation of strain VL749 with plasmid pJZ110.

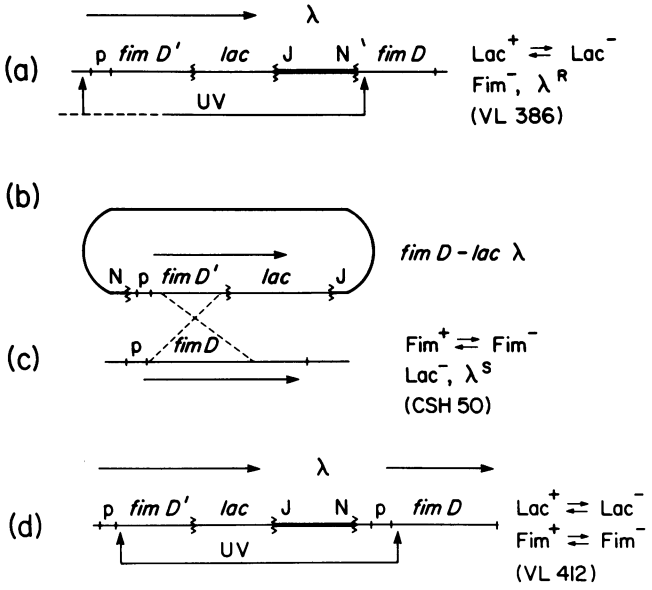

FIG. 1. Construction of strain VL412. Strain designations and phenotypes are indicated at right. The lambda prophage in operon fusion strain VL386 (a) was UV induced to obtain a low-frequency transducing lysate. Some of the phage particles would be expected to contain the intact *fimD-lac* operon fusion along with an active *fimD* promoter as shown by dotted line and arrow (a). Reintegration of the phage (b) into strain CSH50 (c) results in construction of merodiploid strain VL412 (d), which contains both the *fimD-lac* operon fusion and the normal *fimD* operon. UV induction of the merodiploid strain gives rise to a specialized, high-frequency transducing lysate due to flanking regions of DNA homology as indicated by arrows (d).

A plasmid minipreparation was made from strain HB101(pJZ110) (32) by the rapid boiling method of Maniatis (19) and used to transform strain VL749 (phase-locked Lac$^-$). Transformants were selected on MacConkey lactose agar containing 40 μg of ampicillin per ml and then restreaked on the same medium to determine whether Lac phase variation was occurring.

Construction of strain VL751. Strain VL751 is a derivative of strain VL664 that has been cured of the *fimD-lac* λ specialized transducing phage. This strain was constructed by UV induction of strain VL664 and subsequent plating of survivors onto MacConkey lactose medium. Lac$^-$ derivatives were tested for immunity to λ *c*I *b*2 and λ *c*I *h*80. One Lac$^-$ λ sensitive colony was saved (VL751).

RESULTS

Construction of merodiploid strain VL412. A low-frequency transducing lysate was obtained from UV induction of strain VL386 (*fimD-lac* λ) as a result of λ excision via random, illegitimate recombination (Fig. 1). Phage titers were routinely 1×10^5 to 4×10^5 PFU/ml. When the diluted phage lysates were spread with CSH50 (Δ*lac* Fim$^+$) cells on 5-bromo-4-chloro-3-indolyl-β-D-galactoside agar, we obtained a mixed phage lysate: ~40 to 60% large white plaques, ~18 to 25% small white plaques, ~7 to 20% small light blue plaques, and ~9 to 17% slightly larger and more intense blue plaques. The differences in the size and color of the plaques is a reflection of the fact that different phage have packaged different amounts of DNA, depending on the point of excision through illegitimate recombination. We also noted occasional lysogens emerging, presumably from integration of the fusion-bearing phage into the homologous *fimD* region of the chromosome since the λ is lacking its normal attachment sites. λ immune, Lac$^+$ lysogens were isolated, and one strain constructed in this manner (strain VL412) was used in further experiments (Fig. 1). This strain exhibited both Fim$^+$ ⇌ Fim$^-$ and Lac$^+$ ⇌ Lac$^-$ phase variation, demonstrating construction of an *fimD*$^+$ *fimD-lac* merodiploid.

Isolation of specialized phage containing the *fimD-lac* operon fusion. Excision of the λ prophage from strain VL412 resulted in the isolation of a more homogeneous population of phage progeny, because excision in this strain can occur via homologous recombination (Fig. 1). Phage titers were routinely 3×10^9 to 6×10^9 PFU/ml. When CSH50 cells were infected with this high-frequency transducing lysate, λ immune Lac$^+$ lysogens were isolated, all of which were *fimD*$^+$ *fimD-lac* merodiploids. Thus all phage that are induced from VL412 are specialized phage that transduce the operon fusion.

Genetic analysis of merodiploid strains. Since the *fimD*$^+$ *fimD-lac* merodiploid strains exhibited both Fim and Lac switching phenotypes, we wished to determine whether the two phase variations were independently expressed. To assess simultaneously the Lac and Fim phenotypes of individual colonies, we performed a filter immune assay on colonies lifted from lactose indicator agar (Fig. 2). Control strains CSH50 (Δ*lac fim*$^+$) and VL386 (*fimD-lac*) gave Lac$^-$ Fim$^+$ or Fim$^-$ colonies and Lac$^+$ or Lac$^-$ Fim$^-$ colonies, respectively (data not shown). Lac$^+$ and Lac$^-$ colonies of merodiploid strain VL752 are indicated in Fig. 2; the immunoblot of the identical plate shows that, for a given colony, any of four possible phenotypes is possible (Lac$^+$ Fim$^+$, Lac$^+$ Fim$^-$, Lac$^-$ Fim$^+$, and Lac$^-$ Fim$^-$). These results demonstrate that phase variation of Lac and Fim phenotypes is noncoordinate and independent.

We constructed merodiploid strains by other means to confirm the *cis*-dominant phenotype of the switch. F′ factors carrying either the *fim*$^+$ genes or the *fimD-lac* operon fusion were introduced into recipients carrying either the *fimD-lac* operon fusion or *fim*$^+$, respectively (see above). In these merodiploid strains (VL768 and VL773), we also observed that Lac$^+$ ⇌ Lac$^-$ and Fim$^+$ ⇌ Fim$^-$ phase variation occurred in a noncoordinate manner. There did not appear to be any noticeable differences in the switching frequency of the strains whether the *fimD-lac* operon fusion was carried on the chromosome (VL768) or on the F′ factor (VL773). These results corroborate the finding that the phase variation of Lac and Fim phenotypes is noncoordinate and independent.

Reversibility of Lac and Fim phenotypes in merodiploid strains. To determine whether the Lac and Fim phenotypes were reversible, cells from single colonies of each of the four Lac Fim phenotypes from strain VL768 (*recA fimD-lac* F′ Tn*10 fim*$^+$) were diluted and respread to test the offspring for Lac phenotype (by reaction on 2,3,5-triphenyl tetrazolium chloride lactose indicator agar) and for Fim phenotype (by yeast agglutination). These experiments were done to determine whether each of the other three phenotypes could again be obtained through respreading. The rates of phase transition obtained (Table 2) are generally in agreement with those reported previously for Fim phase transitions of *E. coli* B (4) and for Lac phase transition of *E. coli* VL361, our original *fim-lac* fusion derivative (9). The single "mutation" rates obtained for Fim and Lac transitions were slightly higher for switching from negative to positive than for switching from positive to negative, again in agreement with previously

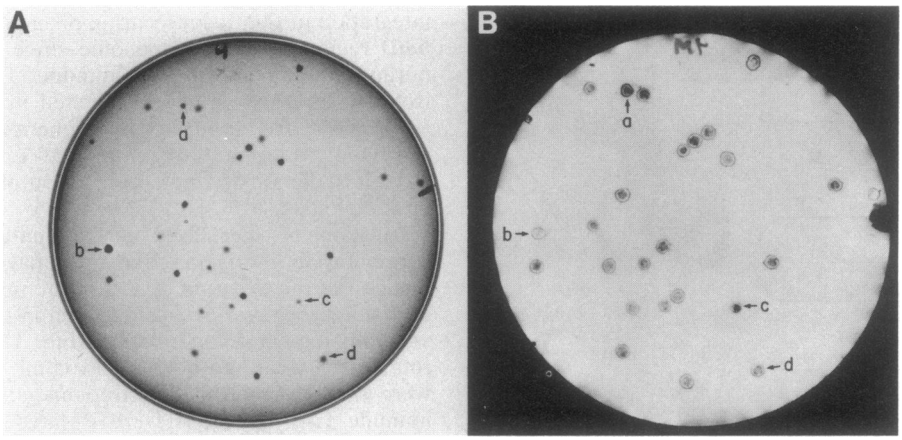

FIG. 2. Merodiploid analysis. Results are shown for strain VL752, an *fimD*$^+$ *fimD-lac* merodiploid strain. The Lac colony phenotype as displayed on a MacConkey lactose indicator plate is shown in panel A (dark colonies are Lac$^+$, light colonies are Lac$^-$). A nitrocellulose filter containing colonies lifted from this plate is shown in panel B. The filter immune assay (see the text) detects differences between Fim$^+$ and Fim$^-$ colonies (dark and light reaction, respectively). This figure demonstrates that four possible phenotypes can be observed in the merodiploid strain: (a) Lac$^+$ Fim$^+$, (b) Lac$^+$ Fim$^-$, (c) Lac$^-$ Fim$^+$, and (d) Lac$^-$ Fim$^-$.

reported results (4, 9). We noted no other differences in phase transition rates, regardless of the starting phenotype of the colony. From each individual plating, each of the three other phenotypes were observed in the population. The frequency of a double switching event (i.e., of both Lac and Fim phenotypes switching) was typically observed at a lower frequency. These experiments were corroborated by using another merodiploid strain, VL752 (data not shown).

Phenotypic characterization of the specialized phage that transduces the operon fusion. High-frequency transducing lysates can be obtained from UV induction of strain VL412 as well as from UV induction of other *fimD*$^+$ *fimD-lac* λ lysogens, obtained through the transduction of CSH50 with λ412. When diluted phage lysates were plated with CSH50 cells (Δ*lac*) on 5-bromo-4-chloro-3-indolyl-β-D-galactoside agar, two plaque phenotypes were noted: strongly positive for β-galactosidase (dark blue) and weakly positive (light blue). Lac$^+$ lysogens were obtained from infecting recipient cells with phage from dark blue plaques, whereas Lac$^-$ lysogens were obtained from infecting recipient cells with phage from light blue plaques; all such lysogens were doubly switching, *fimD*$^+$ *fimD-lac* merodiploids.

When phage from plaques of each phenotype (dark blue and light blue) were isolated and respread, most plaques remained the same phenotype, but some switching occurred (Fig. 3). Thus, the *cis*-active phase switch, as well as the operon fusion, had been cloned on the specialized phage.

Physical characterization of the specialized transducing phage that carries the phase variation control region. Since DNA rearrangement has been shown to be responsible for phase variation of *Salmonella* sp. flagellar antigens (28) and of gonococcal pili (21), we next examined the phage DNA from each plaque type (light blue and dark blue). When the DNA was digested with either *Eco*RI or *Bam*HI, identical restriction patterns were obtained (data not shown), demonstrating the absence of either deletions greater than 400 base pairs or gross rearrangement, associated with the different plaque phenotypes.

Construction and complementation of phase-locked Lac$^+$ λ lysogens of strain VL584. When strain VL584 (Δ*fim*) was lysogenized with specialized transducing phage λ412, almost all of ~2,000 lysogens exhibited Lac$^+$ ⇌ Lac$^-$ phase variation. Nevertheless, we also obtained two Lac$^+$ lysogens that remained phase locked upon subsequent passage. We considered two possibilities for this occurrence: (i) the *cis*-active switch controlling the operon fusion was mutated in some way so that switching could not occur, or (ii) the newly constructed strain lost, at the time of lysogenization, a *trans*-active permissive factor necessary for switching to occur.

To test these two possibilities, we performed complementation and reversion tests. First, we transferred an F′ factor containing the *fim* gene region into the phase-locked strain to see whether the phase-locked mutation could be complemented in *trans*. Exconjugates, selected as described above and then tested on MacConkey lactose medium, all demonstrated the unlocked (Lac$^+$ ⇌ Lac$^-$) phenotype. Phase variation occurred in either *rec*$^+$ or *recA* strain background for every exconjugate tested (approximately 20 exconjugates per mating experiment). Next, we found that when the strains were cured of the F′ by treatment with acridine orange, the phase-locked condition of Lac expression returned, and that the on or off state of Lac reflected the most recent Lac phenotype of the parent before curing.

We determined whether other *trans*-active permissive factors known to be involved in other phase variation systems could complement the phase-locked state. The genes coding for a number of different DNA invertases (*hin*, *gin*, *cin*, and *pin*) have been shown to be functionally interchangeable (14, 15, 26). Specifically we tested the *hin* gene, which codes for the DNA recombinase active in phase variation of *S. typhimurium* flagellar antigens. The phase-locked Lac$^-$ strain VL749, found to be complementable in *trans* by F′ Tn*10* *fim*$^+$, was transformed with pJZ110, a plasmid containing the *hin* gene region from *S. typhimurium* (32). None of the Ap^r transformants demonstrated Lac phase variation, indicating that no complementation had occurred.

TABLE 2. Rates of Fim and Lac phase transition

Starting phenotype		Single mutation rate	
Fim	*Lac*	*Fim*	*Lac*
+	+	$5.52 \times 10^{-3} \pm 2.6 \times 10^{-3}$	$1.02 \times 10^{-2} \pm 0.28 \times 10^{-2}$
+	−	$4.20 \times 10^{-3} \pm 0.86 \times 10^{-3}$	$2.11 \times 10^{-2} \pm 0.40 \times 10^{-2}$
−	+	$3.74 \times 10^{-2} \pm 0.48 \times 10^{-2}$	$1.06 \times 10^{-2} \pm 0.63 \times 10^{-2}$
−	−	$3.20 \times 10^{-2} \pm 0.30 \times 10^{-2}$	$2.7 \times 10^{-2} \pm 0.57 \times 10^{-2}$

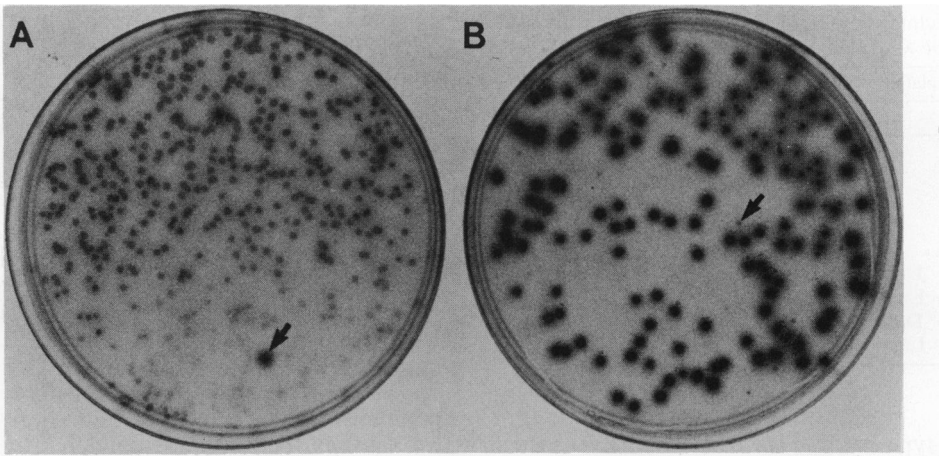

FIG. 3. Plaque phase variation. Individual phage from dark blue and light blue plaques were isolated, diluted, and replated onto a lawn of CSH50 (Δ*lac*) cells. Most of the plaque-purified phage remained the same plaque phenotype, but some switching of plaque phenotypes was noted. Arrows point out plaques whose phage have switched to the alternate plaque phenotype.

Genetic characterization of the *trans*-active factor. We next determined whether the *trans*-active permissive factor, shown to be absent in the phase-locked strain VL664, was originally present on the specialized λ transducing phage or in the parent Δ*fim* strain VL584. To test these two possibilities, VL664 was cured of λ to give strain VL751. Strains CSH50 (*fim*$^+$), VL584 (Δ*fim*), and VL751 (Δ*fim*, λ$^+ \rightarrow$ λ$^-$) were then used as recipients in transduction with λ lysates obtained from UV induction of both switching and nonswitching lysogens. The permissive factor was never encoded by the specialized λ phage, regardless of origin, nor by strain VL751, but was (originally) in strain VL584 (Table 3).

To confirm by an independent technique that the *trans*-active permissive factor was neither on the λ nor in strain VL751, we passaged phage obtained from a switching lysogen (VL412) and nonswitching lysogens (VL664 and VL749) through CSH50 and VL751. The results (Table 4) showed plaque switching on strain CSH50, which has the *trans*-active permissive factor, but not on strain VL751, which is mutant for the *trans*-active permissive factor.

DISCUSSION

We are interested in the molecular genetics of phase variation of type 1 fimbriae in *E. coli*. We have previously shown, using an *fim-lac* operon fusion construction, that fimbria expression is under transcriptional control (9). More recently we positioned a λ prophage adjacent to the operon fusion, mapped its location in the chromosome, and determined its transcriptional orientation (13). This strain, VL386, has provided us the opportunity to determine the location of the switch element relative to the operon fusion by allowing the mobilization of the fusion on a specialized λ phage. Using a mixed phage lysate obtained from UV-induction of VL386 to infect CSH50 (Δ*lac* Fim$^+$) cells, we were able to isolate merodiploid strains that exhibited Lac$^+ \rightleftharpoons$ Lac$^-$ and Fim$^+ \rightleftharpoons$ Fim$^-$ phase variation. Phenotypic analysis of these strains showed that phase variation of Lac and Fim phenotypes is noncoordinate, independent, and reversible. We conclude that the phase variation-controlling region must be duplicated in the merodiploid strain and be acting in *cis*. (A switch acting in *trans* would have driven Fim and Lac together such that their expression would have been coordinate.) Thus the *cis*-active phase variation switch must be directly adjacent to, or part of, the promoter of the *fimD* operon (as well as the promoter of the operon fusion).

When specialized phage obtained from UV induction of the *fimD*$^+$ *fimD-lac* merodiploid strains were plated on CSH50 (Δ*lac* Fim$^+$) cells, two plaque types were noted: dark blue and light blue. Lysogens obtained from either the dark or light plaques demonstrated initial phenotypes of either Lac$^+$ or Lac$^-$, respectively. Since Lac$^-$ lysogens were obtained from the light blue plaques, it is likely that the weakly positive β-galactosidase reaction in the plaque was the result of read-through from a λ promoter rather than expression from the *fimD* promoter. The occurrence of phase variation on the specialized phage during lytic infection confirmed that the phase variation control region had been cloned with the operon fusion.

In *N. gonorrhoeae* phase variation of pilus antigens in-

TABLE 3. Switching activity in lysogens obtained from transduction of recipient strains CSH50, VL584, and VL751 with various *fimD-lac* lysates

λ Lysate	Integration into:			Presence of switch factor on λ phage:	
	CSH50 (*fim*$^+$)	VL584 (Δ*fim*)	VL751 (Δ*fim*)	*cis* Acting	*trans* Acting
λ412 (Lac$^+ \rightleftharpoons$ Lac$^-$)	Switching	Switching	Locked (Lac$^+$)	+	−
λ648 (Lac$^+ \rightleftharpoons$ Lac$^-$)	Switching	Switching	Locked (Lac$^+$)	+	−
λ649 (Lac$^+ \rightleftharpoons$ Lac$^-$)	Switching	Switching	Locked (Lac$^+$)	+	−
λ664 (Lac$^+$)	Switching	Switching	Locked (Lac$^+$)	+	−
λ749 (Lac$^-$)	Switching	Switching	Locked (Lac$^-$)	+	−

TABLE 4. Switching activity of plaques during passage of various *fimD-lac* λ lysates on strains CSH50 and VL751

Lysate	Initial plating		Second passage	
	CSH50	VL751	CSH50	VL751
λ412	Dark blue		Dark and light blue	
	Light blue		Dark and light blue	
λ664	Dark blue		Dark and light blue	
λ749	Light blue		Dark and light blue	
λ412		Dark blue		Dark blue
		Light blue		Light blue
λ664		Dark blue		Dark blue
λ749		Light blue		Light blue

volves a cassette-type mechanism of DNA rearrangement whereby silent copies of genes encoding pilus antigens are moved into expression sites (20, 21). Unlike *N. gonorrhoeae*, *E. coli* cells do not generate antigenically distinct fimbriae during phase transitions (4). It appears unlikely that the phase variation that we observe on the specialized phage during lytic infection is due to a cassette-type mechanism, unless such a mechanism can occur in the absence of phage integration into the bacterial chromosome. In *S. typhimurium* alternate expression of H1 and H2 flagellar antigens involves the inversion of a 995-base-pair segment of DNA that is flanked by 14-base-pair inverted repeat sequences (28). Restriction analyses of phage DNA from light and dark blue plaques with either *Eco*RI or *Bam*HI would not necessarily be expected to detect an inversion involving such a small segment of DNA. The bacterial DNA contained on the specialized transducing phage has been subcloned into pBR322 and used in Southern blot analyses of Fim^+ and Fim^- chromosomal digests. Thus far, no DNA rearrangements have been detected (J. Abraham, unpublished observations). These results could indicate that phase variation of type 1 fimbriae involves rearrangement of very small segments of DNA, similar to that of the gonococcal opacity factor (29), or that phase variation of type 1 fimbriae occurs via an entirely different mechanism not involving DNA rearrangement.

When the specialized λ phage was transduced into a Δ*fim* strain (VL584) known to be deleted of both *fimD* and its phase variation control region, we obtained $Lac^+ \rightleftharpoons Lac^-$ switching lysogens, confirming our results that the *cis*-active switch resides on the specialized phage. We also obtained rare Lac^+, nonswitching lysogens. Complementation tests performed with *recA* recipients and an F′ factor containing the *fim* gene region revealed that the phase-locked condition of Lac expression could be alleviated, regardless of whether they were locked in Lac^+ or Lac^- states. When strains were cured of the F′, the strains became phase locked in either Lac^+ or Lac^-, depending on their state just before F′ curing. Therefore, there did not appear to be a mutation in the *cis*-active switch, but rather in a gene encoding a *trans*-active permissive factor, which must map near the *fim* operon at 98 min. Moreoever, the phase variation process is *recA* independent, confirming previous findings (9). These results also demonstrate more conclusively that the phase variation control region cloned on the specialized phage is adjacent to the operon fusion and is a *cis*-acting element. If the phase variation control regain were elsewhere and acting in *trans* to regulate fimbrial phase variation, as an activator or repressor, we should have obtained phase-locked strains in either an off (mutated activator) or an on (mutated repressor) condition, but not both. We were able to isolate both off and on phase-locked strains, thereby ruling out a *trans*-active phase variation control region.

Because the phase-locked strains were not the result of a mutated switch, but rather appeared to arise from loss of a *trans*-active permissive factor that could be supplied by F′ *fim*$^+$, we next determined whether this factor had been lost from the specialized transducing phage itself or from the recipient strain during lysogenization. We distinguished between these two possibilities by analyzing the specialized transducing phage obtained from the phase-locked strains and by analyzing a derivative of the phase-locked strain that had been cured of its λ prophage (VL751). Our results demonstrated that only the *cis*-active element is present on the specialized transducing phage, whereas the *trans*-active permissive factor is present in strains CSH50 (*fim*$^+$) and VL584 (Δ*fim*), but not in strain VL751 (Table 3). Although strain VL751 was derived from strain VL584, the former strain must have acquired a new mutation in the gene coding for the *trans*-active permissive factor. Further experiments (Table 4) confirmed that the *trans*-active permissive factor is encoded neither by the λ nor by strain VL751. These results are in agreement with our finding that when a recombinant plasmid containing all of the bacterial DNA from the λ phage is introduced into the phase-locked strain, no complementation occurs (unpublished observations).

The presence of both *cis*- and *trans*-active factors is reminiscent of the phase variation system of *S. typhimurium*. Its invertible segment of DNA controls the flagellar phase variation by a *cis*-acting mechanism (32) and also contains the *hin* gene, which encodes a site-specific invertase (28), an enzyme that acts in *trans* to control the orientation of the invertible DNA segment. It has previously been shown that the genes coding for a number of different DNA invertases (*hin*, *gin*, *cin*, and *pin*) are functionally interchangeable (14, 15, 26). Nevertheless, we found no complementation of our fimbrial phase-locked state by using a *hin*-containing plasmid. This result was not particularly surprising since it had previously been shown that Hin^- phage that contained the rest of the flagellar phase variation apparatus could not be complemented in an *E. coli* background (28). In addition, Southern blot analyses with *hin* DNA as a probe failed to demonstrate homologous DNA in the *E. coli* chromosome (30). A similar inversion system, which can complement *gin* mutations of Mu, has recently been discovered in some, but not all, K-12 strains of *E. coli* (25). The *gin* complementing function has been mapped within element *e*14 (which has properties of a defective prophage) located at min 25 on the *E. coli* chromosome (31). The complementing function, encoded by the *pin* gene, is responsible for the inversion of an 1,800-base-pair segment of DNA called the P-region (25), but no function has yet been correlated with orientation of the P-segment. Since our *trans*-complementing function maps at around 98 min on the *E. coli* chromosome and is not complemented by *hin*, it is highly unlikely to have any correlation to *pin*.

The *trans*-complementing factor described in this publication also does not appear to be related to the recently reported 23-kilodalton protein from a clinical isolate of *E. coli*, which acts in *trans* to repress the level of fimbriation (23, 24); *hyp* mutants of *E. coli* still exhibit phase variation of type 1 fimbriae (P. E. Orndorff, personal communication). Thus the genetic regulation of fimbriation is complicated. We expect that the phase-locked strains will be useful in understanding the molecular details of this complex process; these strains have already enabled us to clone the gene for

the *trans*-active factor and to prepare switch DNA in either positive or negative orientation for sequencing.

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