Phase Variation of Type 1 Fimbriae in *Escherichia coli* Is Under Transcriptional Control

Abstract. *An operon fusion of the* lac *genes to those required for synthesis of type 1 fimbriae (pili) has been achieved in a K12 strain of* Escherichia coli *lysogenized by the bacteriophage mu* d *(Ap^r,* lac*). Synthesis of* β*-galactosidase, therefore, reflected* pil *gene transcription and was used as a probe of fimbrial regulation. Expression of the operon fusion was found to oscillate, demonstrating that phase variation between fimbriate and nonfimbriate states is under transcriptional control. The transition rates from fimbriate to nonfimbriate were 1.05 × 10^{-3} per bacterium per generation and from nonfimbriate to fimbriate, 3.12 × 10^{-3} per bacterium per generation.*

The attachment of many Gram-negative bacteria to host cells is mediated by protein organelles on the bacterial surface known as fimbriae or pili (*1*). These surface organelles bind to stereospecific, complementary receptors on susceptible host tissues and thereby overcome the cleansing effects of mucosal secretions, peristalsis, and fluid flow. Type 1 fimbriae mediate mannose-sensitive binding of bacteria to erythrocytes (*2*), epithelial cells (*3*), and leukocytes (*4*). The capacity to colonize mucosal surfaces correlates with the degree of fimbriation (*3, 5*) whereas the resistance to phagocytosis parallels lack of fimbriation (*6*).

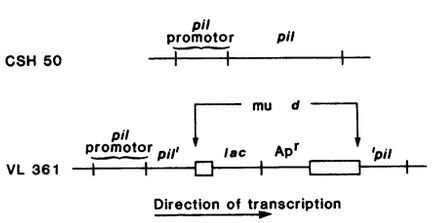
Most of what is known about the genetic regulation of fimbriation has been provided by studies of Brinton and coworkers (*7*), who determined that there are at least three *pil* genes, located at minute 98 of the *Escherichia coli* chromosome. Since fimbriae consist of multimers of a single polypeptide (*2*), one of the *pil* genes must be the structural gene and the others must be needed for regulation, subunit assembly, or both. The most distinctive mechanism that regulates fimbrial synthesis and expression is the all-or-none phase variation between fimbriation and nonfimbriation (*7*), with a rate of oscillation as high as one per thousand bacteria per generation (*7*).

Unfortunately, the difficulty of quantifying the degree of fimbriation, the fact that the expression of fimbriae depends upon protein synthesis followed by subunit assembly, and the fact that fimbriae often provide bacteria a marked growth advantage (*8*) have made it difficult to study the regulation of fimbrial synthesis by conventional techniques. One possible approach to circumventing these problems is the use of the in vivo operon fusion technique (*9*). Coupling of the genes for fimbrial synthesis to the *lacZ* structural gene would place the synthesis of β-galactosidase under the transcriptional control of the fimbrial promoter. This report describes the application of this technique to the study of the regulation of fimbrial genes, and the data show that the observed phase variation in fimbrial synthesis is under transcriptional control. Moreover, the ease of assaying β-galactosidase, the *lacZ* gene product, permitted a precise determination of the rates of phase variation.

The *E. coli* K12 strain (CSH50 from the Cold Spring Harbor Laboratory collection) (*10*) produces type 1 fimbriae and flagella but not sex pili, and the genes of the *lac* operon are deleted (Δ*lac*). The bacteriophage used for the one-step fusion was mu *d* (Ap^r, *lac*), as the lysogen MAL103 (*9*). The phage contains a gene determining ampicillin resistance (Ap^r) and *lac* genes without the *lac* promoter. Bacteria were cultured at 30°C.

The general strategy was to insert bacteriophage mu *d* (Ap^r, *lac*) within a *pil* gene, thereby simultaneously preventing synthesis of fimbriae and placing the inserted *lac* operon under transcriptional control of the *pil* promoter (Fig. 1). If mu *d* is in the proper orientation, such a *pil-lac* operon fusion would result in the synthesis of β-galactosidase as if it were fimbrial protein. The assay of β-galactosidase activity should serve as a direct measurement of the activity of the *pil* gene to which the *lac* genes have been fused. From about a thousand independent lysogens screened, one irreversible nonfimbriate Lac$^+$ mutant (VL361) of strain CSH50 was isolated (Fig. 1).

To ascertain that bacteriophage mu *d* (Ap^r, *lac*) was inserted in only one gene and that this gene was one needed for fimbrial expression, P1 bacteriophage was propagated on the Pil$^+$ parent,



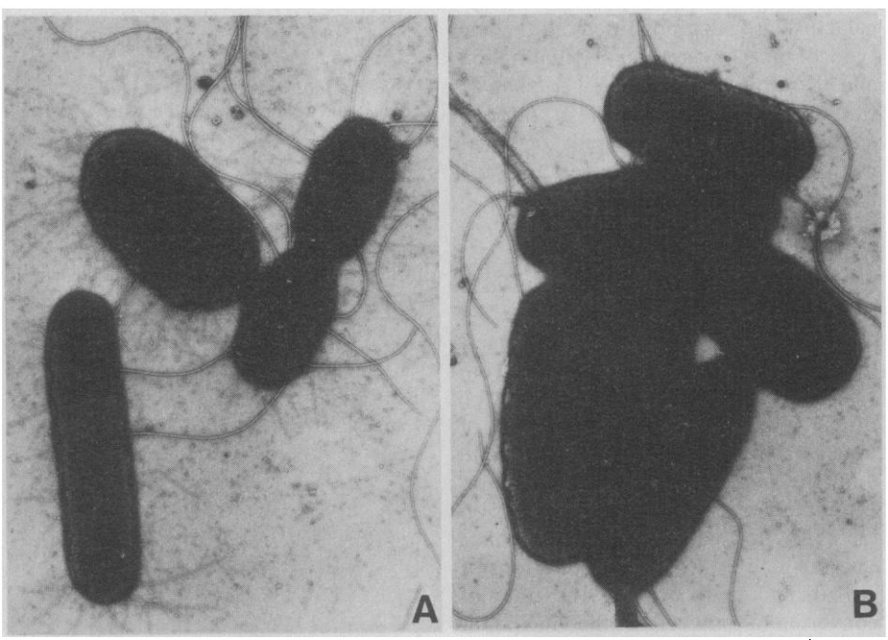


Fig. 1. (Left) Fusion of *lac* to the *pil* operon. Strain CSH50 (Pil$^+$, Δ*lac*) was infected with bacteriophage mu *d* (Ap^r, *lac*) and lysogens were selected which were Lac$^+$ and ampicillin-resistant (*9*). Potential Pil$^-$ isolates, with the *pil* gene cleaved into *pil'* and *'pil* segments, were then selected by flooding with guinea pig erythrocytes (2 percent suspension) the agar plates containing the lysogens and cloning nonhemadsorbing colonies. One clone, VL361, was shown to be a stable Pil$^-$ mutant by lack of pellicle formation in broth (*7*) and lack of fimbriae, as measured by mannose-sensitive hemagglutination (*12*) and electron microscopy (*12*), despite multiple, serial passages in broth. Pil$^+$ could be reconstituted in strain VL361 by transducing it with P1 bacteriophage propagated on strain CSH50. All Pil$^+$ transductants became Ap^s Lac$^-$. (Right) Electron micrographs of *E. coli* strains stained with phosphotungstic acid (*12*). (A) Strain CSH50, showing fimbriae on each bacterium. (B) Fusion strain *pil-lac* VL361, derived from strain CSH50; fimbriae are absent (×20,400).

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CSH50, and was then used to transduce VL361 to Pil$^+$ (*10*). Such transductants were selected by an outgrowth in static aerobic broth (*11*) and had type 1 fimbriae as judged by electron microscopy and mannose-sensitive hemagglutination assays (*12*). Of nine Pil$^+$ transductants, all became ampicillin-sensitive (Ap^s) and Lac$^-$. As further confirmation, tetracycline resistance (Tc^r) was transduced to strain VL361 from strain CSH50 containing the Tn10 transposon positioned randomly in the chromosome. Of 14 Tc^r transductants that were screened as Ap^s, all simultaneously became Lac$^-$ and Pil$^+$. These results suggest that the only mu insertion in the mutant strain VL361 is in a *pil* gene.

To show that β-galactosidase synthesis was actually reflecting *pil* expression, it was necessary to demonstrate that enzyme production would follow the characteristics of normal fimbrial production. In *E. coli* the property of phase variation, the all-or-none oscillation between the expressed and nonexpressed state of a gene, is limited to expression of fimbriation (although similar to flagellar synthesis in *Salmonella*). When the fusion strain was grown on lactose-MacConkey agar, β-galactosidase production (as determined by the coloration of the colonies) was either present or absent, and many colonies were sectored. As would be predicted for a system regulated by phase variation, single Lac$^+$ colonies gave rise to both Lac$^+$ and Lac$^-$ colonies, the majority being Lac$^+$. Single Lac$^-$ colonies acted similarly except that the majority of the offspring were Lac$^-$. In all cases the colonies remained Pil$^-$ and Ap^r. Thus, the production of β-galactosidase demonstrated the same type of phase variation as fimbriation and therefore could be used to analyze the transcriptional control of fimbrial synthesis. Also, of hundreds of Lac$^+$ colonies examined, all gave off Lac$^-$ colonies at equal frequency, an indication that the mu insertions were not translocating to other operons at a detectable rate under the conditions used in these studies.

Given the stability of the mu insertions, the rate of phase transition from Lac$^+$ to Lac$^-$ (and the reverse) in the fusion strain should reflect the rate of transition from fimbriate to nonfimbriate (and the reverse) in the parent strain. Determination of these rates depends on the ability to follow a single cell (either Lac$^+$ or Lac$^-$) through g generations of growth and then measure the ratio of converted to total cells in the final population (Fig. 2). Table 1 lists the calculated rates of transition of bacteria grown at 30°C (g was found to be 28 for both Lac$^+$ and Lac$^-$ colonies). The state of the original cell is assumed to be that of the original colony used to inoculate the agar surface. The rate of conversion of Lac$^-$ and Lac$^+$ (nonfimbriate to fimbriate) was three times that of Lac$^+$ to Lac$^-$ (fimbriate to nonfimbriate). The fact that g was the same for both Lac$^+$ and Lac$^-$ colonies indicated that the apparent rate of the phase variation was not influenced by differences in growth rates of the two phases.

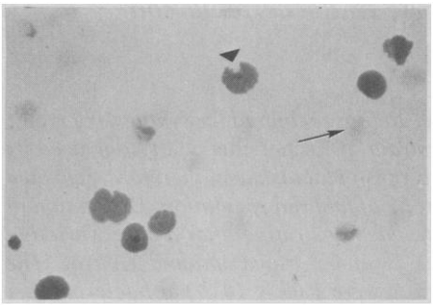

Fig. 2. β-Galactosidase production of phase variant colonies of strain VL361. Bacteria were plated in a layer of soft nutrient agar, grown overnight at 30°C, and stained with bromo-2-naphthyl-β-D-galactoside (0.4 mg/ml) and fast blue RR (1 mg/ml) (*18*). Dark colonies are in positive phase and are synthesizing enzyme. Light colonies are more difficult to see because of lack of staining, are in negative phase, and do not synthesize enzyme. Arrowhead demonstrates a negative sector arising out of a positive colony; arrow, a negative colony.

Table 1. Phase transition rates from Lac$^+$ to Lac$^-$ and Lac$^-$ to Lac$^+$ of single cells of strain VL361. Rates of phase variation were calculated by a modification of the method of Enomoto and Stocker (*19*). The *pil-lac* fusion strain was inoculated onto a lactose-MacConkey plate and, after overnight growth, single Lac$^+$ and Lac$^-$ colonies were streaked to single colonies onto LB agar plates. After overnight growth, individual blocks of agar, each bearing a single colony, were cut out and transferred to 5 ml of minimal medium in separate test tubes and vigorously agitated. Dilutions were made to calculate viable counts and to determine the ratios of Lac$^-$ cells and Lac$^+$ cells to total cells (Fig. 2). 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 g is the number of generations of growth from a single cell to the number of cells within a colony.

State	M/N	Rate of phase transition
Lac$^+$	3/147	7.3×10^{-4}
Lac$^+$	3/116	1.1×10^{-3}
Lac$^+$	15/460	1.2×10^{-3}
Lac$^+$	25/622	1.4×10^{-3}
Lac$^+$	11/480	8.2×10^{-4}
Mean		$1.05 \pm 0.28 \times 10^{-3}$
Lac$^-$	11/207	1.9×10^{-3}
Lac$^-$	13/208	2.1×10^{-3}
Lac$^-$	38/450	3.0×10^{-3}
Lac$^-$	60/650	3.3×10^{-3}
Lac$^-$	9/61	5.3×10^{-3}
Mean		$3.12 \pm 1.35 \times 10^{-3}$

I conclude that phase variation of *E. coli* is under transcriptional control and may be similar genetically to phase variation of *Salmonella* flagella. Silverman *et al.* (*13*) have found that the on-and-off state of flagellin production is regulated by a DNA insertional element, which is capable of two orientations in the genome. In the "on" mode, the flagellin promotor is positioned to allow transcription of the flagellin structural gene, whereas in the "off" mode the direction of the promotor is antiparallel and no transcription occurs. Like phase variation for flagella, that for fimbriae is independent of the *rec*A product; a *rec*A *E. coli* strain in our collection (*14*) exists in both fimbriate and nonfimbriate states (*15*). The rates of phase variation in *Salmonella* are virtually identical to that found for fimbriae and even show a similar asymmetry in direction (*16*). We have cloned the fusion on lambda phage and in merodiploid studies have found the switch to be active in *cis* only (*17*).

It is likely that phase variation is important in the pathogenicity of *E. coli* and other bacteria that possess type 1 fimbriae. *Proteus mirabilis*, which exhibits phase variation of fimbriae, is much more likely to cause pyelonephritis in experimental ascending infections if it is in the fimbriate phase (*5*). In contrast the nonfimbriate phase is more virulent when the route of inoculation is hematogenous (*6*), probably because leukocytes as well as epithelial cells recognize fimbriae via mannose receptors (*4*). Insight into the genetic control of specific bacterial adherence factors such as *E. coli* fimbriae may permit new approaches to studies of bacterial virulence.

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Modulation of Parallel Fiber Excitability by Postsynaptically Mediated Changes in Extracellular Potassium

Abstract. *Field potentials and extracellular potassium concentration ($[K^+]_o$) were simultaneously monitored in the molecular layer of the rat cerebellar cortex during stimulation of the parallel fibers. The synaptic field potential elicited by stimulation was reduced by several methods. Reduction of synaptic field potentials was accompanied by a marked increase in the excitability of the parallel fibers. This change in excitability was related to the degree of extracellular K^+ accumulation associated with parallel fiber stimulation. These findings support the proposal that increases in $[K^+]_o$ associated with activity in postsynaptic elements can modulate the excitability of presynaptic afferent fibers.*

Neuronal communication at chemical synapses in the mammalian central nervous system is often assumed to be unidirectional, proceeding from pre- to postsynaptic elements. However, several studies indicate that ionic changes associated with postsynaptic activity may modulate the excitability of presynaptic elements (*1*, *2*). In contrast to invertebrate systems, where simultaneous pre- and postsynaptic intracellular recording is possible (*1*), pre- and postsynaptic responses in the mammalian brain are difficult to study simultaneously. The cerebellum, with its laminar organization and well-defined pre- and postsynaptic electrophysiological responses, allows for such an analysis. The excitability (*3*) of the parallel fibers (PF's) of the cerebellar cortex is enhanced by the reduction of activity in postsynaptic elements (*4*). This implies that an event secondary to postsynaptic activity can influence presynaptic fiber excitability. In these experiments, the excitability of the PF's was studied before and after reduction of surround synaptic activity and was shown to be related to evoked changes in extracellular potassium concentration ($[K^+]_o$). The findings support the hypothesis that increases in $[K^+]_o$ secondary to activity in postsynaptic elements can modulate the excitability of afferent PF's and perhaps affect information processing of the cerebellum.

Adult male Wistar rats were anesthetized with urethane (150 mg per 100 g of body weight). The cerebellar vermis was exposed (*4*) and superfused with warmed (38°C), oxygenated Ringer solution (*5*). Potassium-sensitive microelectrodes (*6*) were used to record $[K^+]_o$ and field potentials from the same location. Glass-coated tungsten stimulating microelectrodes (tip exposures, 5 to 20 μm) were located 0.3 to 1.5 mm from the recording electrode. Recordings were obtained within 70 μm of the cerebellar surface (*7*).

The field potential elicited by a stimulus applied to the folial surface (*8–10*) consists of an initial triphasic potential (P1, N1, P2 in Fig. 1A) representing the synchronous volley of PF action potentials, followed by a negative potential (N2) corresponding to the synaptic current generated by monosynaptic activation of dendrites in the molecular layer. In response to a 50-Hz, ten-pulse stimulation train (Fig. 1B), latency is reduced in the second and next few field responses, but subsequent responses show increased latency with corresponding amplitude (P1 peak to N1 peak) reductions. During the stimulation train, $[K^+]_o$ increases from a baseline of 3.0 m*M* to a

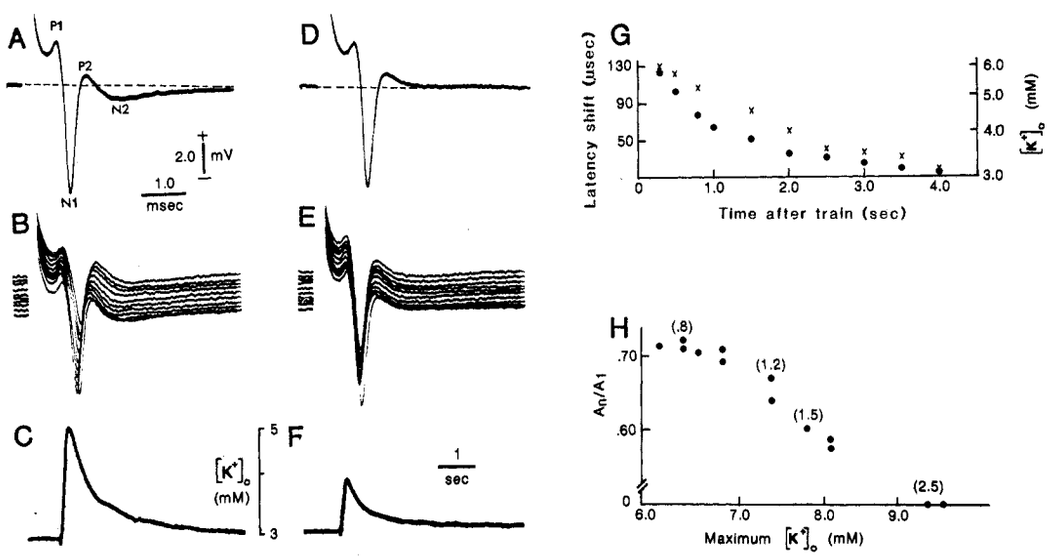

Fig. 1. Field potentials and $[K^+]_o$ changes elicited by PF stimulation in normal Ringer solution (A to C) and 10 m*M* Mg^{2+}-Ringer solution (D to F). (A) On-beam field potential. (B) Consecutive field responses elicited by a 50-Hz, ten-pulse stimulation train. (C) The simultaneous increase in $[K^+]_o$ for response in (B) recorded with a K^+-sensitive microelectrode. (D) Field potential recorded from the same location as in (A to C), but in the presence of 10 m*M* Mg^{2+}. The N2 synaptic potential is obliterated. (E) Field responses elicited by the 50-Hz, ten-pulse stimulation train in the presence of 10 m*M* Mg^{2+} exhibit enhanced frequency-following. (F) The $[K^+]_o$ signal simultaneous to the responses in (E). (G) Latency shift of the field potential (×) and $[K^+]_o$ (●) as a function of the interval after completion of a 50-Hz, 25-pulse stimulation train. $[K^+]_o$ is plotted on a logarithmic scale. (H) A_n/A_1 ratio elicited by 50-Hz, 25-pulse stimulation train plotted as a function of the maximum $[K^+]_o$ elicited by the stimulation train. Points on the graph were obtained by intermittent cerebellar superfusion with a 2 m*M* Mn^{2+}-Ringer solution. Amplitude (in millivolts) of the N2 potential is indicated by numbers in parentheses. $[K^+]_o$ is plotted on a logarithmic scale. Calibrations in (A) pertain also to (B), (D), and (E).

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