JOURNAL OF BACTERIOLOGY, July 1973, p. 299-306
Vol. 115, No. 1

Genetic and Biochemical Investigation of the *Escherichia coli* Mutant *hfl-1* Which is Lysogenized at High Frequency by Bacteriophage Lambda

MARLENE BELFORT AND DANIEL L. WULFF

Department of Molecular Biology and Biochemistry, University of California, Irvine, California 92664

Received for publication 3 April 1973

The *Escherichia coli* mutant *hfl-1* is lysogenized at very high frequency by bacteriophage lambda. The normal requirement for the λcIII gene product in the establishment of repression is not observed in *hfl-1* strains. These phenotypic characteristics are specified by a single locus at 82.5 min on the *E. coli* map in extremely close proximity to the *purA* gene, cotransduction frequencies ranging from 97 to 100% depending on the particular *purA* marker used. The lactose operon is shown to function normally in this strain, and there are also no demonstrable differences in ribonucleic acid polymerase activity or cyclic-adenosine monophosphate levels. Alterations in the cell envelope are indicated by a slight rifamycin resistance, which is reversible by pretreating the cells with ethylenediaminetetraacetic acid, and by a resistance to penicillin and a sensitivity to high concentrations of sodium dodecyl sulfate. It is not known whether this change in cell surface is the primary lesion, or a pleiotropic effect of some more basic metabolic shift.

An infecting temperate bacteriophage particle multiplies in its bacterial host in one of two ways. In a productive lytic infection the phage deoxyribonucleic acid (DNA) molecule directs the synthesis of numerous gene products and replicates autonomously, culminating in the release of many intact progeny phage particles. In a lysogenic infection the DNA becomes incorporated in the host chromosome, after which the only lambda gene to be expressed is the λcI gene, which represses lytic functions. In this latter case the inserted bacteriophage DNA, or prophage, replicates as a unit with the bacterial DNA. The decision between lysis and lysogeny depends upon the interaction of several lambda phage genes. Lysogeny is favored by the timely synthesis of adequate amounts of repressor protein, the λcI gene product, whose synthesis is under the positive control of the λcII and λcIII gene products. This repressor molecule is also responsible for the maintenance of the stable lysogenic state. The lytic pathway is also delicately controlled and is favored by conditions which facilitate phage DNA replication, so swamping out the effectiveness of the repressor. A prophage may be induced to enter productive growth when for any reason the repressor ceases to act. These events are well summarized in a recent review by Echols (4).

The frequency with which an infecting lambda particle enters the lysogenic rather than the lytic pathway depends not only on phage genotype but also on a variety of cellular metabolic conditions (12) and on bacterial genotype, as shown by the isolation of various bacterial mutants which alter the typical course of infection of a particular phage particle. Examples of such host mutants are those with a defective cyclic-adenosine monophosphate (AMP) system, which are lysogenized less readily than are wild-type cells (10, 11, and Belfort and Wulff, manuscript in preparation), and the *hfl* mutants which undergo *h*igh *f*requencies of *l*ysogeny on infection with wild-type lambda (2 and B. Rolfe, personal communication). Previous studies have shown that the requirement for the λcIII gene as a positive control element for repressor synthesis is relieved in *hfl* strains (2) and that λc17, a clear mutant with a new rightward promoter, abortively infects *hfl-1* strains (Belfort and Wulff, unpublished data).

In this communication we describe the ge-

netic mapping of the *hfl-1* mutation. This has enabled us to ascribe the entire spectrum of phenotypic properties of *hfl-1* to a single locus and has allowed us to study the mutation at the biochemical level in new strains, so avoiding spurious effects resulting from unlinked secondary mutations in the original *hfl-1* strain. We have examined ribonucleic acid (RNA) polymerase, the functioning of the lactose operon, cyclic-AMP levels and various characteristics of the cell surface in order to define the primary biochemical lesion of the *hfl-1* mutation.

MATERIALS AND METHODS

Bacterial strains. The derivatives of *Escherichia coli* K-12 employed are listed and characterized in Table 1. The direction of transfer and points of origin of the Hfr strains used in this study are given in Fig. 1, together with locations of the markers used for mapping. Hft strains not listed in Table 1 are from the collection of B. Low (J. Bacteriol., in press).

Media. Tryptone broth (TB) was routinely used for growth of cells (1.0% tryptone, 0.5% NaCl, supplemented with 1.5% agar for solid media). Luria broth (LB) (16) was used for cultures in P1 transduction, whereas LB broth without glucose (LBB) was used for Hfr matings. Tris calcium (TC) buffer used in transductions contains 0.01 M tris(hydroxymethyl)-aminomethane (Tris), pH 7.4, 0.4% NaCl, 2.5 mM $CaCl_2$, and 0.01% gelatin. Minimal medium 56, minus carbon source (18), was used as the routine buffer, as well as for selection of recombinants for nutritional markers. For solid media, Agar agar #3 (Consolidated Laboratories, Chicago), autoclaved separately, was added to a final concentration of 1.0%; the carbon source (glucose, or succinate when selecting for the *fdp*$^+$ marker) was added at 0.4%, amino acids and bases at 20 μg/ml and vitamins at 0.1 μg/ml. For selection against most Hfr donor strains, streptomycin sulfate (Calbiochem) was added to the plates at 100 μg/ml, whereas rifamycin (Calbiochem) was used at 100 μg/ml to select against strains KL228, and KL14. Tryptone yeast extract (TYE) plates (8) were used for maintenance of Hfr strains and also for spot testing selected colonies for the Hfl character. EMBO plates containing 0.1% yeast extract were prepared as described by Gottesman and Yarmolinsky (9) and used for replica-plating to determine the Hfl phenotype.

Conjugation. Hfr strains were maintained and colonies checked in plate matings for their ability to transfer early markers as described by Low (J. Bacteriol., in press). Log-phase cultures of donor Hfr strains (1 ml at about 5×10^7 cells per ml in LBB broth) and F$^-$ *hfl-1* strain UC2014 (1 ml at 5×10^8 cells per ml in LBB broth) were mixed and incubated at 37 C in a 250-ml flask without shaking. A Rif^R derivative of strain UC2014, UC2042, was used with Hfr strains

TABLE 1. *Strains of E. coli K-12 used*

Strain	M[a]	Hfl	Other relevant chromosomal markers[b]	Source or reference
UC4185	F$^-$	+	*ser*$^-$, *trp*$^-$, *leu*$^-$, *ilv*$^-$, *lys*$^-$, *str*$^-$	D. Wulff collection
UC2014	F$^-$	−	*ser*$^-$, *trp*$^-$, *leu*$^-$, *ilv*$^-$, *lys*$^-$, *pan*$^-$, *pyr*$^-$, *str*$^-$	Derivative of UC4185 (2)
UC2042	F$^-$	−	*ser*$^-$, *trp*$^-$, *leu*$^-$, *ilv*$^-$, *lys*$^-$, *pan*$^-$, *pyr*$^-$, *rif*$^-$, *str*$^-$	Derivative of UC2014 Spontaneous mutation to Rif^R
D/4	Hfr	+	*metB*$^-$, *str*$^+$	W. F. Dove
DF1100	HfrH	+	*fdp-1*, *str*$^+$	Fraenkel (6)
KG20	F$^-$	+	*argH*$^-$, *his*$^-$, *pro*$^-$, *thi-1*, *ampA*$^-$, *purA*$^-$, *str*$^-$	CGSC[c] (5)
AB3584	F′	+	*thi-1*, *metC56*, *valS7*, *xyl-7*, *galK2* *lacY*$^-$, *tfr-5*, *tsx-57*, *ara-14*, (λ$^+$)	CGSC (23)
ES4	F$^-$	+	*purA45*, (*mtl-2*, *xyl-7*, *mal*$^-$, *galK6* *lac*$^-$, *tsx*$^-$, *ton*$^-$, *φ80*^{R}, *str*$^+$)[d]	CGSC (5)
AT2535	F$^-$	(+)[d]	*pyrB59*, *argH1*, *his-1*, *purF*$^-$, *rel*$^-$, *mtl-2*, *xyl-7*, *malA*$^-$, *str*$^-$, *tsx*$^-$, λ^R, *sup-48*	CGSC (22)
AN169	F$^-$	+	*ubiA420*, *argH*$^-$, *str*$^-$	Young (24)
UC2156	F$^-$	+	( )[e]	Derived from ES4 by transduction to *pur*$^+$ with P1 grown on UC4185
UC2157	F$^-$	−	( )	Derived from ES4 by transduction to *pur*$^+$ *hfl-1* with P1 grown on UC2014

[a] M, Mating type.
[b] The heavily underscored markers are those used in transduction studies.
[c] CGSC, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.
[d] λ*h*80cIII plates intermediate in turbidity between that typical of an *hfl*$^+$ and an *hfl*$^-$ strain, but AT2535 is assigned the Hfl$^+$ phenotype.
[e] ( ), Denotes all sugar and non-nutritional markers of strain ES4.

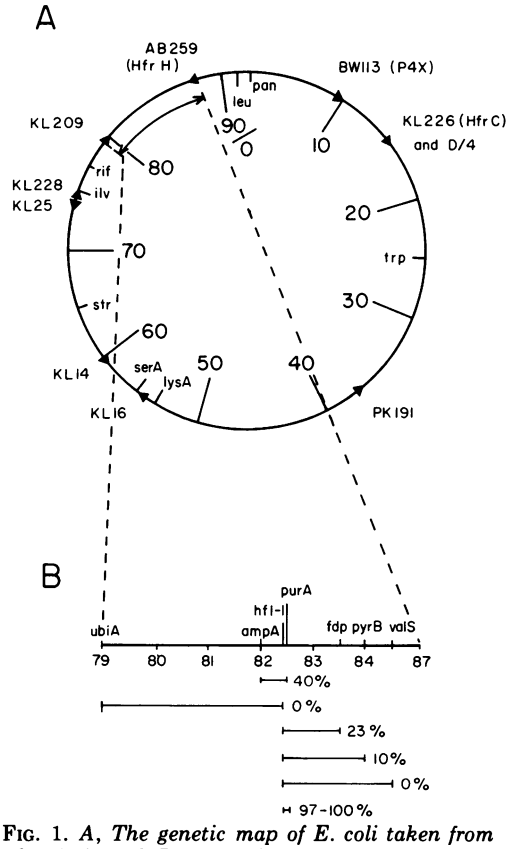

FIG. 1. *A, The genetic map of E. coli taken from Taylor (21) and B. Low (J. Bacteriol., in press). Positions of relevant genetic markers are given within the circle. The origins of Hfr strains are given by arrowheads indicating direction of transfer and are labeled outside the circle. B, Amplification of the region between the origins of HfrH and P10. Markers shown are those used in transduction mapping. Hfl-1 is located at 82.5 min. Bars indicate regions being transduced and percentages above each bar represent cotransduction frequencies. The placement of hfl-1 to the left of purA is tentative.*

KL228 and KL14. Mating was interrupted at 40 min by diluting 10-fold into 56-buffer and agitating vigorously in a vortex mixer for 1 min. Cells were filtered onto a membrane filter (0.22 μm pore size; Millipore Corp.), washed with 56-buffer, suspended into buffer, and plated at the appropriate dilution onto recombinant-selective plates. As controls, unmated donors and recipients were treated exactly as above and plated at the lowest dilution.

P1 transductions. P1 stocks were grown on the appropriate donor strain using the soft agar plating method, as described by Eriksson-Grennberg (5). The recipient strain was grown at 30 C overnight in LB broth and diluted 10- to 20-fold to approximately 2×10^8 cells per ml into TC buffer. A mixture of 0.2 ml of bacteria in TC buffer and 0.2 ml of P1 at 2×10^9/ml in TC buffer was incubated at 37 C for 30 to 40 min for adsorption, and plated onto transductant-selective plates at the appropriate dilution. Minimal media plates were incubated at 37 C for 2 to 3 days. When strain AB3584(ts) served as recipient, the P1-infected cells were plated onto TYE plates, which were then incubated at 30 C for 4 h to allow for phenotypic expression of the *valS*$^+$ marker. Plates were then shifted up to 41 C and incubated overnight. Uninfected controls were employed in parallel in all transductions.

Testing selected colonies for the Hfl-phenotype. Recombinants were screened for the Hfl phenotype by inoculating large freshly grown colonies with an applicator stick into a drop of broth or buffer in a test tube or in the well of a plastic disposable tray. They were then evenly spread onto TYE plates and droplets of the appropriate phage were applied. All lambda lysates used for these tests were grown on strain UC4185(P1) in order to allow the phage to overcome restriction in transductants lysogenic for P1. Droplets of a phage suspension at about 5×10^5/ml were added from a 1-ml tuberculin syringe, and the plates were incubated at 33 or 37 C overnight. The Hfl$^-$ phenotype is distinguishable on the basis of the turbid plaques formed by λcIII as opposed to the clear plaque morphology and higher plating efficiency on Hfl$^+$ strains. Phage $\lambda c17$ was also used to distinguish the Hfl character since it does not grow efficiently on *hfl-1* strains (Belfort and Wulff, unpublished data). Lambda hybrids with the host-range of $\phi 80$ ($\lambda h80$) were used with some recipients which were sensitive to *h*80, but not to *h*λ strains. Phage λimm^{434} were used to test the phenotype of strains lysogenic for λ. Table 2 summarizes the phage strains used to test

TABLE 2. *Phage strains used for checking Hfl phenotype*

Bacterial strain	Marker(s) of interest	Phage used for spot test
UC4185 and derivatives	All, see Table 1	λcIII[a] and λc_{17}[b] (Hfr crosses) λcIII.P1 and λc_{17}.P1 (transductions)[c]
AB3584	*valS*	λimm^{434}cIII.P1[d]
ES4 and derivatives	*purA*	λcIII.P1 and λc_{17}.P1
AN169	*ubiA*	λcIII.P1 and λc_{17}.P1
DF1100	*fdp-1*	λcIII.P1 and λc_{17}.P1
AT2535	*pyrB*	λcIII*h*80.P1[e] and λ^+h80.P1[f]
KG20	*ampA, purA*	λcIII*h*80.P1[e] and λ^+h80.P1[f]

[a] All cIII mutants used here are derivatives of $\lambda cIII_{co2}$ (from J. Parkinson).

[b] λc_{17} from W. Sly.

[c] The P1 suffix denotes growth of the phage on a P1 lysogen.

[d] Obtained by crossing $\lambda imm^{434}c^+$ with λcIII.

[e] Obtained by crossing λ^+h80 with λcIII.

[f] Obtained from H. Echols. Plates with reduced efficiency on *hfl-1* and used as a check instead of $\lambda c_{17}h80$, which does not grow on a P1 lysogen.

the phenotype of the various bacterial recipients.

The replica plating method was sometimes used for a cruder survey of the presence of Hfl colonies in transduction and conjugation experiments. This method is based on the fact that *hfl-1* strains are lysogenized efficiently by λcIII, while *hfl*$^+$ strains are not. Small colonies were replica-plated onto EMBO plates seeded with 10^7 cells of the appropriate λcIII phage and incubated at 33 C for 10 to 16 h. Hfl$^-$ colonies stand out as round, pale pink replicas among a background of darker, irregularly shaped colonies. In re-examining these putative Hfl$^-$ colonies by the screening procedure described above, 97% were verified as Hfl$^-$ by using *h*λ and 90% by using *h*80 phage.

β-Galactosidase assays. Cells were grown at 37 C in 56-minimal medium without $CaCl_2$ and containing 0.2% glycerol, 0.1% Casamino Acids, and all the nutritional requirements of strain UC2014. At a cell density of 5×10^7/ml, β-galactosidase was induced by adding isopropyl-β-D-thiogalactoside (IPTG) (Mann Biochemicals) to a final concentration of 5×10^{-4} M, and at the appropriate times 1-ml samples were transferred to an equal volume of 0.1 M phosphate buffer containing 0.01% sodium deoxycholate (Fischer) and 0.005 ml of toluene. The assay was based on the hydrolysis of orthonitrophenyl-β-D-galactoside (ONPG) (Calbiochem) (13). Glucose was added at 0.2% together with the IPTG for catabolite repression studies and adenosine 3′:5′-cyclic adenosine monophosphate (cAMP) (Calbiochem) was added concomitantly to observe reversal of catabolite repression. One unit of enzyme is defined as that amount of enzyme which hydrolyzes 1 nmol of ONPG in 1 min under the assay conditions used (13). In order to normalize units to cell number, viable cell counts were determined on TB agar at each time-point.

RNA polymerase extraction and assay. DNA-dependent RNA polymerase was extracted from both UC4185 and UC2014 cultures exactly as described by Burgess (3) through the steps of sonic oscillation, DNAse treatment, ammonium sulfate fractionation, and diethylaminoethyl-cellulose chromatography. Assays, based on the polymerization of adenosine triphosphate, cytidine triphosphate, guanosine triphosphate (P and L Biochemicals), and ^{14}C-uridine 5′-triphosphate (Schwartz radiochemicals) into trichloroacetic acid-insoluble material, were also performed using the Burgess procedure (3). The templates used were calf-thymus DNA (Sigma, type XX) and λDNA (generously supplied by M. Konrad).

Cyclic AMP assays. Cells were grown in TB to stationary phase, and samples of cell cultures, or cell pellets suspended in acetate assay buffer, were sonically treated, the debris was removed by centrifugation, and these supernatant fluids as well as supernatant fluids of the original cultures were treated in a boiling water-bath for 5 min, cooled, and brought to pH 4 with 6 N HCl. Samples were assayed by the procedure of Gilman (7) for their ability to compete with ^{3}H-cAMP for binding to cAMP-binding protein (Nuclear Dynamics, Calif.). Total protein was measured in extracts using the procedure of Lowry et al. (15).

Determination of drug and detergent sensitivities. The given drug or detergent was added at the appropriate concentration to a logarithmic culture and growth was measured through a Klett-tube sidearm of the growth flask. For the rifamycin studies, cells were pretreated with 2×10^{-3} M ethylenediaminetetraacetic acid (EDTA) (14).

RESULTS

Genetic mapping of the hfl-1 locus. Crosses of the *hfl-1* strain UC2014 with different Hfr strains, selecting for various nutritional markers and testing these recombinants for the Hfl character, indicated that *hfl-1* lies in the *leu-ilv* region of the *E. coli* genetic map. Since Hfr strains KL25, D/4, KL226 and BW113 all transfer the *hfl*$^+$ marker, and since strains KL209 and AB259 do not transfer *hfl*$^+$, the *hfl-1* mutation can be localized to the interval defined by the points of origin of strains KL209 and AB259 (79 to 87 min). The inability of strains KL16, PK191, and KL14 to transfer the marker is also consistent with the mutation lying in the 79- to 87-min region.

The results of cotransduction studies done with strains bearing markers in this region are shown in Table 3. An extremely tight linkage with *purA* is indicated by 100% cotransduction of *hfl-1* with the *purA* marker of strain ES4 and 97% cotransduction with the *purA* marker in strain KG20. In this latter cross none of the five transductants which were Hfl$^+$ was Amp$^+$, which best fits a gene order of *ampA-hfl-1-purA*, where *purA* is the *purA* marker of strain KG20. These studies also indicate that three properties of the *hfl-1* mutation, namely the high lysogenic response, lack of a requirement for the λcIII gene product, and abortive infection by λc17, are inseparable by recombination. Further genetic studies for ordering the *hfl* and *purA* loci are in progress.

β-Galactosidase induction. The β-galactosidase system was studied to see if some common regulatory element involved in expression of both lambda and the *lac* operon has been altered by the *hfl-1* mutation. In fact, as can be seen in Fig. 2, β-galactosidase induction and repression is normal in the *hfl-1* strain UC2014, and strain UC2014 overcomes catabolite repression (17) in the presence of cyclic-AMP just as the parent strain UC4185 does.

Measurement of cyclic-AMP levels. Cyclic-AMP is known to have some influence in channeling an infecting lambda particle into the lysogenic pathway (10, 11). The elevated lysogenic response in the *hfl-1* strain UC2014 is, however, not a cyclic-AMP effect, for this strain has wild-type levels of the nucleotide. Cyclic-AMP assays on extracts of both strain UC4185

TABLE 3. *P1-mediated transduction of genes in the 79- to 85-min region of the E. coli map*

Donor *hfl-1*	Recipient *hfl*$^+$	Selected marker	No. of transductants scored		Unselected markers	No. of recombinants	Relative frequency (%)
			Spot test	Replica plating			
UC2014	AN169	*ubiA*$^+$	80	0	*hfl-1*	0	0
					hfl$^+$	80	100
UC2014	ES4	*purA*$^+$	56	250	*hfl-1*	306	100
					hfl$^+$	0	0
UC2014[a]	KG20	*purA*$^+$	180	0	*hfl-1 amp*$^-$	103	57
					hfl-1 amp$^+$	72	40
					hfl$^+$ *amp*$^+$	5	3
					hfl$^+$ *amp*$^-$	0	0
UC2014	DF1100	*fdp*$^+$	76	0	*hfl-1*	18	23
					hfl$^+$	58	77
UC2014	AT2535	*pyrB*$^+$	54	0	*hfl-1*	5	9
					hfl$^+$	49	91
UC2014	AB3584	*valS*$^+$	34	380	*hfl-1*	0	0
					hfl$^+$	414	100

[a] Although UC2014 has an increased resistance to penicillin, it is more sensitive than KG20, and can for mapping purposes be considered *amp*$^-$.

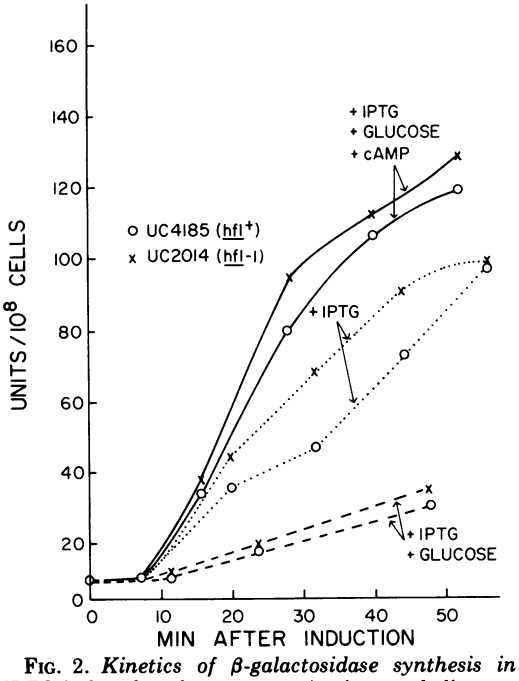

FIG. 2. *Kinetics of β-galactosidase synthesis in IPTG-induced cultures (·····), in catabolite repressed cultures (- - -) and upon reversal of catabolite repression with cyclic AMP (——). All cultures were induced with IPTG at 0 min. Glucose and cyclic-AMP were also added to the appropriate culture vessels at zero time.*

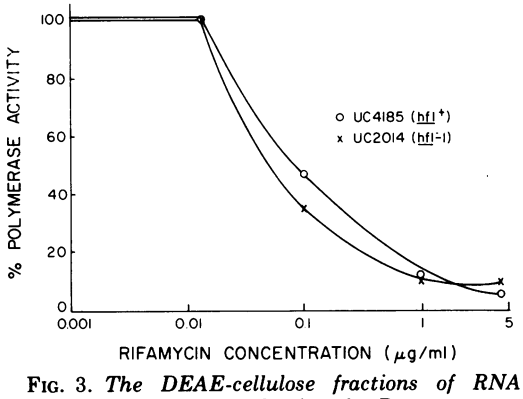

FIG. 3. *The DEAE-cellulose fractions of RNA polymerase were extracted using the Burgess procedure and the assays also performed using his method (3). Lamba DNA was used as template in the above experiment. Calf thymus DNA yielded similar results.*

(*hfl*$^+$) and strain UC2014 (*hfl-1*) reveal intracellular concentrations of 0.2 nmol of cAMP per mg of protein and extracellular levels of 37 nmol per mg. This finding is consonant with the normal inducibility in strain UC2014 of the *lac* operon, a system on which cyclic-AMP is known to exert a profound influence (20).

RNA polymerase studies. Since an infecting λ particle is dependent on the *E. coli* RNA polymerase for transcription of its genes, an altered polymerase could be responsible for the Hfl phenotype. Such a possibility is supported by a slightly increased resistance to rifamycin in both *hfl-1* strain UC2014 and the *hfl-1pur*$^+$ transductant of strain ES4 (UC2157). However, no demonstrable differences in the RNA polymerases from strain UC4185 (*hfl*$^+$) and strain UC2014 (*hfl-1*) could be detected with respect to either patterns of inhibition in the presence of rifamycin (Fig. 3) or specific activity of the

enzyme with lambda or calf thymus DNA as templates. The in vivo resistance to rifamycin is further examined in the following section.

Colicin sensitivity and the resistance of hfl-1 strains to drugs and detergents. The membrane-defective, temperature-sensitive, colicin-tolerant *tol IV* mutant, 483, isolated by Nomura and Witten (19), was shown by B. Rolfe and co-workers (personal communication) to undergo an exaggerated lysogenic response on lambda infection. We therefore studied the susceptibility of *hfl-1* strains to the lethal action of colicins, as well as their sensitivity to various drugs and detergents.

Strains UC2014 (*hfl-1*) and UC4185 (*hfl*$^+$) proved to be equally sensitive to the killing action of colicin E2 over a wide range of concentrations at both 30 and 41 C. There was also no difference in sensitivity to chloramphenicol and spectinomycin. However, both strain UC2014 and strain UC2157, the *hfl-1pur*$^+$ transductant of strain ES4, showed an increased resistance to penicillin (Fig. 4A) and rifamycin (Fig. 5) with respect to the corresponding *hfl*$^+$ strains. Since the in vitro studies revealed no alteration in behavior of the RNA polymerase from the *hfl-1* strain UC2014, the growth-response of strains UC2157 (*hfl-1*) and UC2156 (*hfl*$^+$) in the presence of rifamycin was studied in cells pretreated with EDTA in order to increase membrane permeability (14). The results shown in Fig. 5 indicate that pretreatment with EDTA removes the difference in rifamycin sensitivity between strains UC2157 and UC2156. The finding that *hfl-1* strains are unusually sensitive to high concentrations (2%) of sodium dodecyl sulfate (Fig. 4B) is further suggestive of alterations in the surface properties of *hfl-1* cells. The was no difference in deoxycholate (DOC) sensitivity (Fig. 4B).

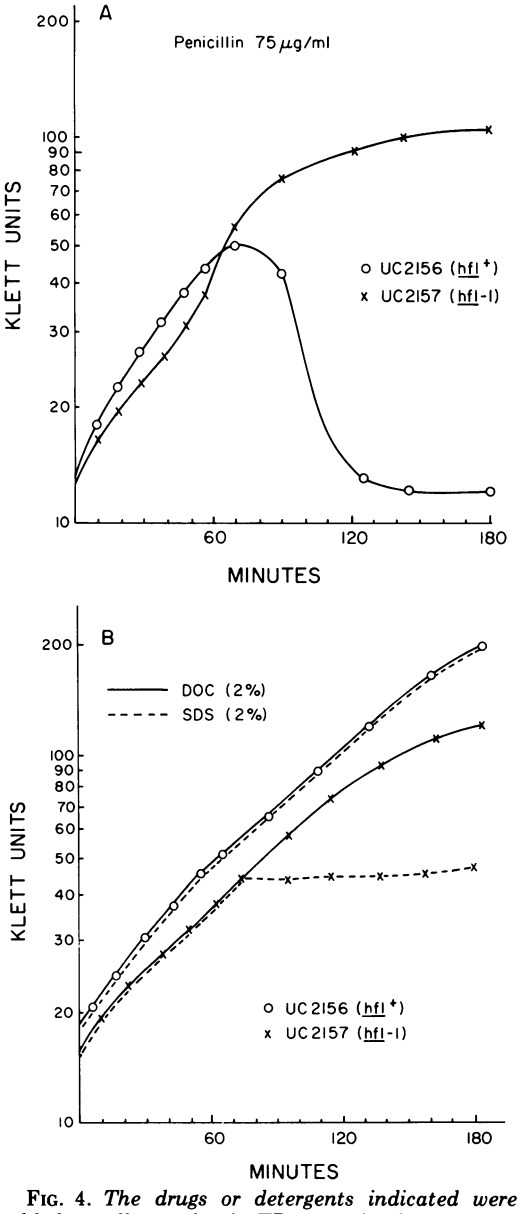

FIG. 4. *The drugs or detergents indicated were added to cells growing in TB at 0 min.* O *represents UC2156 (hfl*$^+$*) and* ×, *UC2157 (hfl-1).*

DISCUSSION

Our results show the *hfl-1* mutation to be very tightly linked to the *purA* locus, cotransuction frequencies ranging from 97 to 100%, depending on which particular *purA* mutant was used as recipient. This places *hfl-1* at approximately 82.5 min on the *E. coli* genetic map.

This mapping study has yielded much useful information. First, it allows one to attribute the entire range of altered responses to infection by bacteriophage lambda, including the high lysogenic response, lack of a requirement for the λcIII gene product, and abortive infection by λc17, to a single mutational event. Second, it has facilitated the construction of strains that are isogenic except for the *hfl* region. Any biochemical differences which then reveal themselves in such strains are assured of being a real property of the mutated *hfl* locus, rather than a consequence of some secondary mutation. Third, the extremely close proximity to the *purA* locus suggests that *hfl-1* might be an altered *purA* gene, or alternatively that there may exist some functional relationship between the two genes. However, since an *hfl-1purA* double mutant retains the Hfl$^-$ phenotype (Gautsch and Wulff, unpublished data), it

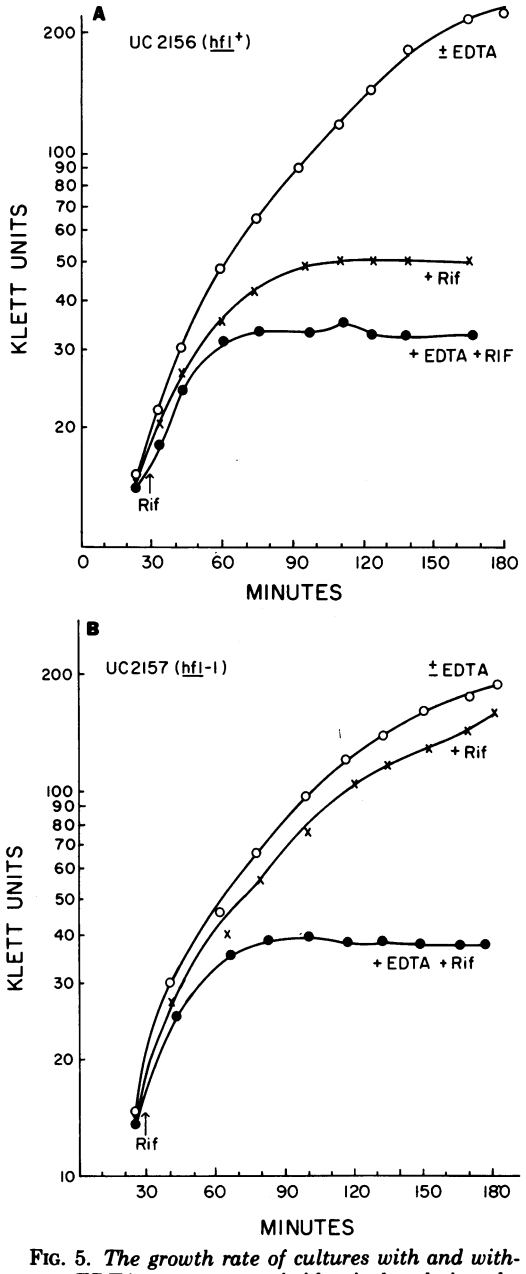

FIG. 5. *The growth rate of cultures with and without an EDTA pretreatment is identical and given by* (○). *The 2-min EDTA treatment was given at 0 time, while rifamycin was added at 30 min at a final concentration of 10 μg per ml.* (×) *denotes growth of untreated cells to which rifamycin has been added, and* (●) *growth of EDTA-treated cells in the presence of rifamycin.*

would seem unlikely that the *hfl* locus lies within the *purA* gene itself.

The specificity with which *hfl-1* strains alter the infective process of lambda is quite striking. In an independent study we have shown that such closely related lambdoid phage as $\phi 80$ or even λimm^{80}, as well as the more distantly related temperate phage like P1, show no shifts in lysogenic response (Belfort and Wulff, submitted for publication). Only those lambdoid phage known to have a lambda cIII gene (λ, 434, and 21) are lysogenized with high frequency. Further, the lactose operon, whose control is reminiscent of that of lambda with respect to negative regulation by repressors and positive inductive control (1), appears to be expressed normally. In this communication we have shown that IPTG induction of the β-galactosidase enzyme of the lactose operon as well as glucose repression and reversal of catabolite repression by cyclic-AMP are similar in *hfl*$^+$ and *hfl-1* strains. Cyclic-AMP levels were also measured since bacterial strains that lack a functional adenyl cyclase enzyme (*cya*$^-$) have been shown to be lysogenized less readily by lambda (10, 11). No differences in cAMP levels were observed in extracts of *hfl*$^+$ and *hfl-1* strains.

In vitro studies of RNA polymerase revealed no differences between *hfl*$^+$ and *hfl-1* strains. The in vivo resistance to low concentrations of rifamycin in *hfl-1* strains proved to be almost completely reversible by pretreating *hfl-1* cells with EDTA, implying that decreased permeability is accountable for the apparent increase in drug resistance. The increased resistance of *hfl-1* strains to penicillin and their greater sensitivity to the detergent sodium dodecyl sulfate are further indications of an alteration in the cell envelope. It is not known whether this change in the cell surface is the primary lesion, or a pleiotropic effect of some more basic metabolic shift. In either event, we are presently unable to explain why the *hfl* mutation should lead to high levels of lysogeny and relieve the requirement for the λcIII gene.

ACKNOWLEDGMENTS

We are thankful to M. Mahoney and K. Kawahara for their competent and cheerful technical help, to E. and C. Domingo for their guidance in isolating RNA polymerase and to M. Konrad for his generous gift of purified lambda DNA.

Thanks are also due to B. Rolfe for communicating his unpublished results, and to B. Bachmann, J. Cronan, D. Fraenkel, and T. Young for sending bacterial strains.

This work was supported by National Science Foundation grant no. NSF-GB 32194 to D.W.

LITERATURE CITED

1. Attardi, G. S., J. Naono, J. Rouviere, F. Jacob, and F. Gros. 1963. Production of messenger RNA and regulation of protein synthesis. Cold Spring Harbor Symp. Quant. Biol. 28:363-372.
2. Belfort, M., and D. L. Wulff. 1971. A mutant of *Escherichia coli* that is lysogenized with high frequency, p. 739-742. *In* A. Hershey (ed.), The bacteriophage lamb-

da. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
3. Burgess, R. R. 1969. A new method for the large scale purification of *Escherichia coli* deoxyribonucleic acid-dependent ribonucleic acid polymerase. J. Biol. Chem. **224**:6160–6167.
4. Echols, H. A. 1972. Developmental pathways for the temperate phage: lysis vs lysogeny. Annu. Rev. Genet. **6**:157–190.
5. Eriksson-Grennberg, K. G. 1968. Resistance of *Escherichia coli* to penicillins. II. An improved mapping of the *ampA* gene. Genet. Res. **12**:147–156.
6. Fraenkel, D. G. 1967. Genetic mapping of mutations affecting phosphoglucose isomerase and fructose diphosphatase in *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. **57**:698–705.
7. Gilman, A. E. 1970. A protein binding assay for adenosine 3′:5′-cyclic monophosphate. Proc. Nat. Acad. Sci. U.S.A. **67**:305–312.
8. Gottesman, S., and J. Beckwith. 1969. Directed transposition of the arabinose operon: a technique for the isolation of specialized transducing bacteriophages for any *Escherichia coli* gene. J. Mol. Biol. **44**:117–127.
9. Gottesman, M. E., and M. Yarmolinsky. 1968. Integration negative mutants of lambda. J. Mol. Biol. **31**:487–505.
10. Grodzicker, T., R. R. Arditti, and H. Eisen. 1972. Establishment of repression in lambdoid phage in catabolite activator protein and adenylate cyclase mutants of *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. **69**:366–370.
11. Hong, J., G. R. Smith, and B. N. Ames. 1971. Adenosine 3′:5′-cyclic monophosphate concentration in the bacterial host regulates the viral decision between lysogeny and lysis. Proc. Nat. Acad. Sci. U.S.A. **68**:2258–2262.
12. Jacob, R., and E. L. Wollman. 1959. Lysogeny, p. 319–351. *In* F. N. Burnet and W. M. Stanley (ed.), The viruses, vol. 2. Academic Press Inc., New York.
13. Kennel, D., and B. Magasanik. 1964. Control of the rate of enzyme synthesis in *Aerobacter aerogenes*. Biochem. Biophys. Acta **81**:418–434.
14. Leive, L. 1965. Actinomycin sensitivity in *Escherichia coli* produced by EDTA. Biochem. Biophys. Res. Comm. **18**:13–17.
15. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. **193**:263–275.
16. Luria, S. E., and J. W. Burrous. 1957. Hybridization between *Escherichia coli* and *Shigella*. J. Bacteriol. **74**:461–476.
17. Magasanik, B. 1970. Glucose effects: inducer exclusion and repression, p. 189–219. *In* J. R. Beckwith and D. Zipser (ed.), The lactose operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
18. Monod, J., G. Cohen-Bazire, and M. Cohn. 1951. Sur la biosynthese de la β-galactosidase (lactase) chez *Escherichia coli*. La specificite de l'induction. Biochem. Biophys. Acta **7**:585–599.
19. Nomura, M., and C. Witten. 1967. Interaction of colicins with bacterial cells. III. Colicin-tolerant mutations in *Escherichia coli*. J. Bacteriol. **94**:1093–1111.
20. Pastan, I., and R. Perlman. 1970. Cyclic adenosine monophosphate in bacteria. Science **169**:339–344.
21. Taylor, A. L. 1970. Current linkage map of *Escherichia coli*. Bacteriol. Rev. **34**:155–175.
22. Taylor, A. L., and M. S. Thoman. 1964. The genetic map of *Escherichia coli*. Bacteriol. Rev. **31**:332–353.
23. Tingle, A., and F. C. Neidhardt. 1969. Mapping of a structural gene for valyl-transfer ribonucleic acid synthetase in *Escherichia coli* by transduction. J. Bacteriol. **98**:837–839.
24. Young, I. G., R. A. Leppik, J. A. Hamilton, and F. Gibson. 1972. Biochemical and genetic studies on ubiquinone biosynthesis in *Escherichia coli* K-12: 4-hydroxybenzoate octaprenyltransferase. J. Bacteriol. **110**:18–25.