FINE STRUCTURE MAPPING, COMPLEMENTATION, AND PHYSIOLOGY OF *ESCHERICHIA COLI hfl* MUTANTS

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ABSTRACT

Six of seven *hfl* mutations of *Escherichia coli* K12, characterized by high frequencies of lysogenization by phage lambda and λ*cIII* mutants, are shown to be tightly linked to, but not within, the *purA* locus. All six *hfl* mutations are recessive to wild type in *hfl*+/*hfl* merodiploids and all lie in a single complementation group, located just counterclockwise from the *purA* locus. All six mutations confer a slightly increased resistance to penicillin and rifamycin and a slightly increased sensitivity to sodium dodecyl sulfate. Some cases of intragenic complementation and intragenic recombination were observed. It is argued that the *hfl*+ gene determines the synthesis of a protein which antagonizes lysogenization by phage lambda. It is further argued that the function of the λ*cIII* gene product is to negate the antagonistic effect of this *hfl*+ protein.

IN 1971 BELFORT and WULFF described a mutant of *Escherichia coli* K12 which was lysogenized with very high frequencies by phage lambda and λ*cIII* mutants. Subsequent studies showed that this mutant, called *hfl-1* for *h*igh *f*requency of *l*ysogeny, was also slightly resistant to penicillin and rifamycin, this latter effect being due to a change in permeability, and slightly more sensitive to sodium dodecyl sulfate (BELFORT and WULFF 1973a). Further studies showed that the host *hfl* mutation was *cIII*-specific, which is to say that it acted as if it supplied a *cIII*-like function in abundance to infecting lambda phage particles (BELFORT and WULFF 1973b). But the mechanism by which it acted was unclear. One possibility was that the *hfl-1* mutation represented a *change* in function of some protein within the host so that it could substitute for the λ*cIII* gene product, much in the way in which certain *leuD* mutations may be suppressed by gene substitution (KEMPER and MARGOLIN 1969). Another possibility was that the *hfl-1* mutation represented a *loss* of function, that is, a mutation from an active protein, which might be called the Hfl+ protein, to an inactive protein. The Hfl+ protein would in some way antagonize the establishment of lysogeny by lambda, although the mode of action of the Hfl+ protein could be quite indirect. According to this second model, it would be further argued that the normal λ*cIII* gene product promotes lysogenization by negating the antagonistic effect of this Hfl+ protein. The first hypothesis, that of a change of function in the *hfl-1* mutant,

predicts that *hfl* mutants should be dominant over the *hfl*+ allele in merodiploids. The second hypothesis, that of loss of function in the *hfl-1* mutant, predicts that *hfl* mutants should be recessive to the *hfl*+ allele in merodiploids.

Another interesting facet of the earlier studies on the *hfl-1* mutation was the extremely tight linkage of *hfl-1* to the *purA* locus (BELFORT and WULFF 1973a). With the *purA* mutation in strain ES4, 100% co-transduction was observed (no recombinants in several hundred transductants) and with the *purA* mutation in strain KG20 there was 97% co-transduction. This raised the possibility that the *hfl-1* mutation lay within the *purA* locus itself.

To answer these questions we isolated several *hfl* mutants and did both fine structure mapping and complementation studies with them. The results indicate that the *hfl* mutation represents a *loss* of function and that the *hfl* gene lies to the left (counterclockwise) of all *purA* mutants tested.

MATERIALS AND METHODS

Strains: Bacterial strains used in this study are derivatives of *Escherichia coli* K12; *hfl*+ strains are described in Table 1, haploid *hfl* strains in Table 2 and merodiploid *hfl* strains in Table 3. The methods used to obtain these strains are described more fully below.

TABLE 1

Properties of hfl+ *bacterial strains*

Strain	Genotype	Source or reference
ES4	F⁻, *purA45*, *mtl-2*, *xyl-7*, *mal*⁻, *galK6*, *lac*⁻, *tsx*⁻, *ton*⁻, $\phi 80^R$	BELFORT and WULFF (1973a)
KG20	F⁻, *argH*⁻, *his*⁻, *pro*⁻, *thi-1*, *ampA*⁻, *purA*⁻, *str*⁻	BELFORT and WULFF (1973a)
AT2535	F⁻, *pyrB59*, *argH1*, *his-1*, *purF*⁻, *rel*⁻, *mtl-2*, *xyl-7*, *malA*⁻, *str*⁻, *tsx*⁻, λ^R, *sup-48*	BELFORT and WULFF (1973a)
KL16	Hfr, *thi-1*	LOW (1968)
KL16-99	Hfr, *thi-1*, *recA*	LOW (1968)
F118/KL132	F⁻, *amp*+, *hfl*+, *purA*+, *pyrB*+/*pyrB31*, *thr-1*, *leu-6*, *proA2*, *his-4*, *thi-1*, *thyA25*, *galK2*, *ara-14*, *xyl-5*, *malA1*, *recA1*, *tsx-33*, *strA31*, *sup-37*	MOUNT, LOW and EDMISTON (1972)
UC4185	F⁻, *ser*⁻, *trp*⁻, *leu*⁻, *ilv*⁻, *lys*⁻, *str*⁻	BELFORT and WULFF (1973a)
UC3013	as UC4185, also *purA1*	UV-induced derivative of UC4185 (this paper)
UC3025	as UC4185, also *purA13*	UV-induced derivative of UC4185 (this paper)
UC3031	as UC4185, also *purA19*	UV-induced derivative of UC4185 (this paper)
UC3064	as UC4185, also *ampA*	*ampA* derivative of UC4185 by P1 transduction using KG20 as donor (this paper)
UC3092	F118/UC3025	F118/KL132 × UC3025 (this paper)
UC3093	F118 *purA13*/UC3025	Spontaneous *Pur*⁻ segregant from UC3092 (this paper)

TABLE 2

hfl *derivatives of strain UC4185*

Column 1* *hfl* strains		Column 2† *hfl purA* strains		Column 3‡ *hfl purA ampA* strains
Strain	*hfl* allele	Strain	*purA* allele	Strain
UC2014	1	UC3035	23	UC3058
UC3413	53			UC3414
UC2168	58	UC3040	28	UC3060
UC2043	59	UC3041	29	UC3061
UC2044	65	UC3043	31	UC3062
UC2169	66	UC3045	33	UC3063
UC2167	29			

* The strains in Column 1 are all *hfl* strains derived from UC4185 by NG mutagenesis, with the exception of strain UC3413. Strain UC3413 is a Trp+ Hfl- Rif- recombinant of the cross Hfr KL16 × UC3403. Strain UC3403 was derived from UC4185 by NG mutagenesis. (All strains have the same growth factor requirements as UC4185 except UC2014, which is also *pyr* and *pan*, and UC3413, which is *trp*+.)

† The strains in Column 2 are all UV-induced *purA* mutants of the corresponding strains in Column 1.

‡ The strains in Column 3 are *ampA purA* derivatives of the corresponding strains in Column 2. They were derived by P1 transduction, using UC3064 as donor and selecting for Amp-. Strain UC3414 was derived from UC3413 by P1 transduction, using strain UC3057 as donor and selecting for Amp-. It is *hfl-53 purA19 ampA*.

Media: TB broth, LB broth, EMBO agar, and TC buffer have been described previously (BELFORT and WULFF 1973a). For isolation of *purA* mutants we used a medium of 56/2 buffer (Low 1973) plus glucose (0.4%) and required supplements. For other phases of this study requiring buffer or minimal medium we used the medium E of VOGEL and BONNER (1956), with or without glucose (0.4%) and required supplements. Supplements were at the following concentrations: amino acids, 20 μg/ml; bases, 10 μg/ml; vitamins, 0.1 μg/ml. Agar agar #3 (Consolidated Laboratories, Chicago), autoclaved separately, was added to a final concentration of 1.0% for solid minimal media. Adenine (10 μg/ml) was added to TB broth and LB broth for growth of Pur- strains. In growing P1 transducing stocks on *purA* mutants, adenine was added to LB plates and LB top agar to a concentration of 40 μg/ml. Penicillin G (potassium salt) was added to a concentration of 75 μg/ml to TB agar or minimal agar for testing the *ampA* marker.

Isolation of additional Hfl- mutants: Seven Hfl- mutants, all deirved by N-methyl-N'-nitro-

TABLE 3

Construction of strains with hfl *markers on the F118 episome*

Strain	Genotype	Method of obtaining *hfl* marker on the episome*		
		Method 1 or 2	P1 donor strain	Recipient strain
UC3097	F118 *ampA hfl-1*/UC3035	1	UC3058	F118/UC3035
UC3095	F118 *amp*+ *hfl-53*/UC3025	2	UC3413	F118 *purA13*/UC3025
UC3096	F118 *amp*+ *hfl-58*/UC3025	2	UC2168	F118 *purA13*/UC3025
UC3098	F118 *ampA hfl-59*/UC3035	1	UC3061	F118/UC3035
UC3099	F118 *ampA hfl-65*/UC3040	1	UC3062	F118/UC3040
UC3101	F118 *ampA hfl-66*/UC3045	1	UC3063	F118/UC3045

* See MATERIALS AND METHODS.

N-nitrosoguanidine (NG) mutagenesis of Hfl+ strain UC4185, were included in the study. Strain UC2014, which contains *hfl-1*, is the original Hfl- mutant (BELFORT and WULFF 1971) and five of the six additional Hfl- mutants were isolated by D. WIEBE in this laboratory by the same method. The remaining Hfl- mutant, strain UC3403, was found among NG-induced Rif- mutants of strain UC4185. It grew very slowly and was crossed with Hfr strain KL16 to yield a fast-growing Trp+ Hfl- Rif- recombinant, designated strain UC3413. The Hfl- and Rif- characters of this strain transduce independently of each other and therefore represent two independent mutational events.

Measurement of percent lysogeny: Stationary phase host strains were centrifuged and resuspended at 2×10^8 cells/ml in 0.02 M $MgCl_2$. Equal volumes of cell suspension and phage in TB broth were mixed and, after 30 minutes absorption at 30°, anti-λ serum was added to a final Kt of 5. The infected cells were diluted and plated on TB plates at 37° by the soft-agar method, using strain C600 as indicator bacteria, to enumerate the number of infective centers. To determine the number of lysogens the diluted infected cells were also plated on EMBO plates which had been seeded with ca. 10^9 λ*cI26* particles per plate. After 48 hours at 30°, the white lysogenic colonies were easily distinguished from the darker non-lysogenic colonies (GOTTESMAN and YARMOLINSKY 1968). All experiments were done with phage carrying the temperature sensitive *cI857* repressor, which is active at 30° but not 37°.

P1 transductions: These were done as described previously (BELFORT and WULFF 1973a), except that recipient strains were first grown in a medium containing medium E, glucose (0.4%), required supplements and 1% LB broth, and transductants were selected on solid medium containing medium E buffer instead of 56 buffer. If transductants which were non-lysogenic for phage P1 were required, the multiplicity of infecting P1 transducing phage particles per recipient cell was reduced from 10 to between 0.1 and 1. (Non-lysogenic transductants are more frequent at low multiplicities of infection (LURIA, ADAMS and TING (1960.) For transduction in which Amp- was the selected marker, TB broth and TB agar plus penicillin was sometimes substituted for minimal medium and minimal agar plus penicillin.

Testing selected colonies for the Hfl phenotype: This was done as described previously using λ*cIII* phage (BELFORT and WULFF 1973a) except that about 200 phage particles in .02 M $MgCl_2$ and bacterial colonies resuspended in TB broth were mixed for 30 minutes at room temperature for adsorption, followed by addition of 1 ml TB top agar and pouring onto small (15 × 60 mm) plates containing TB agar.

Isolation and mapping of purA *mutants:* Strains were grown in supplemented minimal medium to approximately 10^8 cells/ml, irradiated with ultraviolet light to about 1% survival, diluted 10-fold into supplemented minimal medium plus adenine and aerated for 8 hours to allow growth and heterozygote segregation. Mutants with an absolute requirement for adenine are *purA* or *purB*, whereas adenine-requiring mutants in other loci are satisfied by guanine as well (STOUTHOMER, DE HAAN and NIJKAMP 1965). The cultures were washed by centrifugation and suspended in supplemented minimal medium plus guanine (2.5 μg/ml) at 10^7 cells/ml and aerated for two hours. Penicillin was added (75 μg/ml) and the cultures aerated for 18 hours. Survivors were plated on minimal agar containing a limiting amount of adenine (1 μg/ml). Following incubation for 2–3 days, small colonies were transferred to minimal agar containing adenine (10μg/ml) and minimal agar containing guanine (10 μg/ml) with sterile toothpicks. About 10% of the small colonies which survived the penicillin selection had an absolute requirement for adenine when the starting strain was UC4185. However, because of varying degrees of penicillin resistance among the *hfl* strains, adenine-requiring mutants of *hfl* strains represented 1% to less than 0.1% of the small colony survivors of penicillin selection.

Isolation of recA *strains:* To obtain *recA* derivatives of various bacterial strains, the Hfr *recA* strain KL16-99 was mated with appropriate recipient strains in LB broth as described previously (BELFORT and WULFF 1973a), followed by selection for Lys+ Str- or Trp+ Str-. Recombinants were judged to be of the RecA- phenotype if they (1) showed increased UV sensitivity and/or (2) allowed λ*cI26* but not λ*bio*10 to form plaques when used as indicator bacteria (SIGNER, MANLY and BRUNSTETTER 1969).

F-prime matings: F-prime matings were performed on solid media in the following manner:

Freshly grown F-prime and F⁻ colonies were transferred by sterile sticks to 0.15 ml medium E buffer to a concentration of about 10^8–10^9 cells/ml. About 0.02 ml of each suspension was spread (by 0.1 ml pipettes at right angles and crossing each other) on a minimal agar designed to allow only the desired mating result to grow. The new merodiploids appeared as isolated colonies below the area of the cross, and as confluent growth at the junction. The resulting merodiploids were further purified by streaking on the same medium.

It was discovered that F′ *ampA/ampA*+ merodiploids have an Amp⁻ phenotype, which allowed us to select for the *ampA* marker in F-prime matings. However, when selection was for AmpA⁻ in this type of mating, it was found, in most cases, that if the F-prime donor carried *ampA purA* on the episome, no transfer would occur. The reason for this is unknown, but it may be related to a similar problem in P1-mediated transduction (ERICKSSON-GRENNBERG 1968), where *ampA purA* donor strains did not generate *ampA purA* transductants. If the F-prime donor was *ampA purA*+, and selection was for *ampA*, transfer and growth of the resulting merodiploid occurred at normal frequencies.

Charcoal-filtered casamino acids, which contain subminimal amounts of aromatics, were added to selection plates (0.4%) in instances where tryptophan and adenine were used in selection. This decreased the time necessary for merodiploid growth from 2–3 days to 1 day.

Construction of F-prime strains with episomal hfl *markers:* Two different methods were used: In method 1, strains of the form F118/*hfl*-y *purA trp recA*+ were made by crossing F118/132 with the corresponding F⁻ *hfl*-y *purA trp recA*+ strain and selecting for Pur+. These merodiploids were then used as recipients in P1 transduction, using an *ampA hfl*-x *purA* donor P1 phage stock and selecting for Amp⁻. Transductants which were Amp⁻ Hfl⁻ Pur+ were tested for the ability to transfer an F118 *amPA hfl*-x *purA*+ episome to an F⁻ recipient which was *ampA*+ *hfl*-y *purA*+ *trp*+ *recA*, where selection was for Amp⁻ Trp+. Strains with this capability (Table 3) were then used as donor strains to construct merodiploids of the form F118 *hfl*-x/*hfl*-z *recA* for complementation tests.

In method 2 we obtained a rare F118 *purA13/purA13* segregant from an F118/*purA13* merodiploid by the method of Low (1973). The frequency of Pur⁻ segregants was about 1 in 250, but only 1 of 200 Pur⁻ segregants turned out to be a suitable F-prime donor. This F118 *purA13/purA13* merodiploid was then used as a recipient in P1 transduction, using *ampA*+ *hfl*-x *purA*+ donors and selecting for Pur+. Transductants were tested for the ability to transfer an F118 *ampA*+ *hfl*-x *purA*+ episome to an F⁻ recipient which was *ampA*+ *hfl*-y *purA trp*+ *recA*, where selection was for Pur+ Trp+. Strains with this capability (Table 3) were then used as donor strains to construct merodiploids of the form F118 *hfl*-x/*hfl*-z *recA* for complementation tests.

RESULTS

Properties of hfl *strains and linkage to* purA: All six additional Hfl⁻ mutants isolated in this study show increased frequencies of lysogenization upon infection by lambda and lambda *cIII* mutants. However, none shows as large an increase as with the original *hfl-1* strain, UC2014. At an average multiplicity of infection of 0.4 phage per bacterium, λ*cI857cIIIco*$_2$ lysogenized strain UC4185 (*hfl*+) at a frequency of 0.8% and strain UC2014 (*hfl-1*) at 61%. Four of the six additional Hfl⁻ strains (with alleles *hfl-53*, *hfl-58*, *hfl-59* and *hfl-66*) were lysogenized at frequencies ranging from 15% to 39%. The remaining two Hfl⁻ strains were lysogenized at considerably lower frequencies, with strain UC2167 (*hfl-29*) at 3.8% and strain UC2044 (*hfl-65*) at 2.2%. Lambda wild type, λ*cIIIco*$_2$ and λ*c17* form plaques on all these Hfl⁻ strains with reduced efficiency, as had previously been shown with the original *hfl-1* strain (BELFORT and WULFF 1971, 1973b).

Transduction studies with phage P1 of the original *hfl-1* strain UC2014 has shown 100% co-transduction of the *hfl-1* marker with the *purA* marker of strain

TABLE 4

Co-transduction of hfl *markers with* purA *markers in strains ES4 and KG20*

	Donor strain	Percent Pur+ which are Hfl- with recipient strains	
	hfl allele	ES4	KG20
UC2014	1	100	91
UC2167	29	0	
UC3413	53	96	84
UC2168	58	88	83
UC2043	59	96	98
UC2044	65	85	97
UC2169	66	94	77

ES4 and 97% co-transduction with the *purA* marker of strain KG20 (BELFORT and WULFF 1973a). We therefore tested the six additional Hfl- strains for linkage to these two *purA* markers. All but one of the Hfl- mutants were found to be tightly linked to the *purA* locus, with co-transduction frequencies ranging from 77% to 98% (Table 4). The remaining Hfl- mutant, strain UC2167 (*hfl-29*) was unlinked to *purA*. It was lysogenized at a frequency of only 3.8% by *λcI857 cIIIco*$_2$ and has not been characterized further.

The original *hfl-1* mutation, when transferred into strain ES4 by phage P1-mediated transduction, conferred a slight increase in rifamycin resistance and penicillin resistance and a slight increase in sensitivity to sodium dodecyl sulfate (BELFORT and WULFF 1973a). Each of the other five *purA*-linked Hfl- mutations was tested in this regard after transfer to strain ES4. All were found to behave like the *hfl-1* mutation.

Functional independence of the hfl *and* purA *genes:* With such high co-transduction frequencies between the *hfl* locus and the *purA* locus, we envisioned the possibility that the *hfl* mutants lay within the *purA* gene. Yet *hfl* could not represent a mutation to *loss* of the *purA* function, for none of the Hfl- strains required adenine for growth. In addition, of some 50 independently isolated UV-induced *purA* mutants, none showed an Hfl- phenotype. The possibility remained, however, that the Hfl- mutation was to be associated with a *change* of the *purA* function which did not impair the ability of the *purA* gene product to function in the adenine biosynthetic pathway. If this were the case, then mutation of an Hfl- PurA+ strain to PurA- should concurrently result in reversion of the Hfl- phenotype to Hfl+. Such an expectation, however, was not fulfilled: Using penicillin selection, a total of 15 UV-induced adenine-requiring mutants were isolated in five of the six *purA*-linked *hfl* mutant strains. All of these mutants retained their Hfl- phenotypes. Thirteen of these adenine-requiring mutants were tightly linked to the *hfl* locus by P1-transduction and could be classified as *purA* mutants (Table 5). This classification was verified for the two *purA* derivatives of strain UC2014 (*hfl-1*) by assaying for the product of the *purA* gene, adenylosuccinic acid synthetase (LIEBERMAN 1956). Activity was found in extracts of strain UC2014 but not in the two *purA* derivatives of strain UC2014. We were unsuc-

TABLE 5

Isolation of purA hfl *mutants*

	Starting strain *hfl* allele	Number of adenine-requiring mutants isolated	Number with Hfl⁻ phenotype	Number tightly linked to *hfl*-locus by P1 transduction
UC2014	1	3	3	2
UC3413	53	0	0	0
UC2168	58	4	4	4
UC2043	59	1	1	1
UC2044	65	2	2	2
UC2169	66	5	5	4
Total		15	15	13

cessful in isolating adenine-requiring mutants of strain UC3413 (*hfl-53*), but found that an *ampA hfl-53 purA* derivative of this strain obtained by P1-transduction retained the Hfl⁻ phenotype.

Mapping hfl *mutants:* Mapping was by P1-transduction with donors which were *ampA hfl purA*x and recipients which were *amp*⁺ *hfl*⁺ *purA*y, where *purA*x and *purA*y are two different *purA* mutations which are separable by recombination. Selection was for Pur⁺ and distribution of the unselected Hfl and Amp phenotypes was noted. The expected results are shown in Figure 1. If *purA*x lies to the right of *purA*y, most of the Pur⁺ transductants will be Hfl⁻ if the order is *ampA–hfl–purA* and Hfl⁺ if the order is *ampA–purA–hfl*. If *purA*x lies to the left of *purA*y, just the opposite results are expected. (The *purA* mutants were first ordered with respect to *ampA* with reciprocal 3 factor crosses by the method of MARGOLIN (1963). With two *hfl* mutants, *hfl-58* and *hfl-65*, the cross was of the form shown in Case 2 of Figure 1. In both cases a majority of recombinants were Hfl⁺ (Table 6), indicating a map order of *ampA–hfl–purA*. With three *hfl* mutants, *hfl-1*, *hfl-59* and *hfl-66*, the cross was of the form shown in Case 1 of Figure 1. In all three cases a majority of recombinants were Hfl⁻ (Table 6) indicating the same map order: *ampA–hfl–purA*. The distribution of the outside *ampA* marker among Pur⁺ recombinants in these crosses is also consistent with this map order (Table 6). Mutant *hfl-53* was not mapped with respect to *ampA* and *purA*.

We have never observed a recombinational event between the *purA* mutation in strain ES4 and the *hfl-1* locus, even after screening several hundred transductants. We therefore wished to know if the *purA* locus in ES4 lies at the left-hand extremity of the *purA* gene. Unfortunately we were unable to map the ES4 *purA* gene using *ampA* as an outside marker, as we did with the other *purA* mutants, because we were unable to grow a stock of transducing phage on an *ampA* ES4 derivative. However, we could tell if the ES4 *purA* marker lay to the right of the two left-hand most *purA* markers on our map, *purA28* and *purA31*, by crosses using either an *ampA hfl-58 purA28* strain or an *ampA hfl-65 purA31* strain as donor and strain ES4 as a recipient. Selection was for Pur⁺ and, of the unselected markers, a majority were found to be Hfl⁺ in both cases (Table 7).

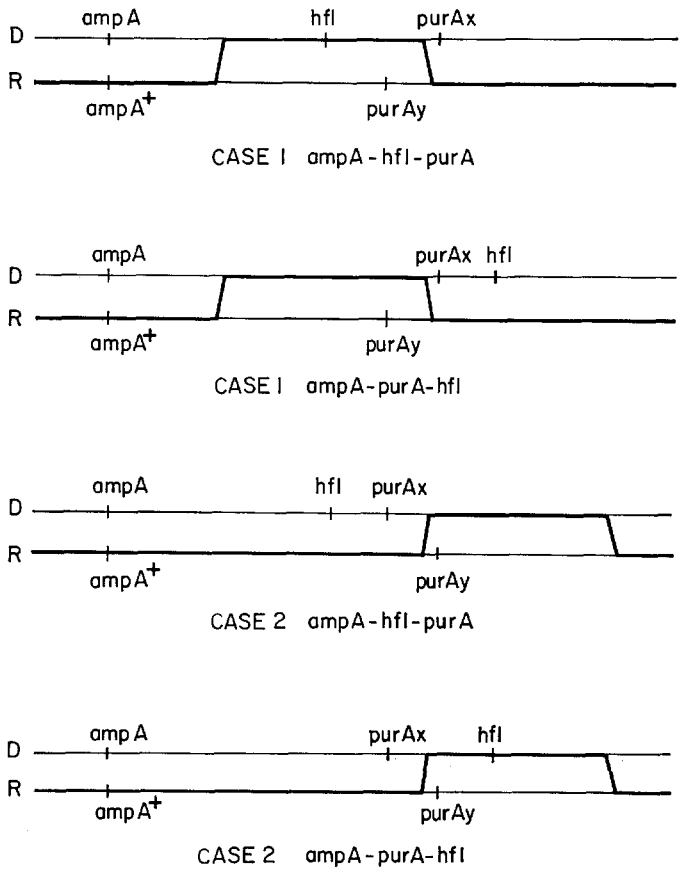

FIGURE 1.—Mapping *hfl* mutants using *purA* donors and recipients. In Case 1 the recipient *purA* allele is closer to *ampA* than the donor *purA* allele. In Case 2 the donor *purA* allele is closer to *ampA*. Mapping by this method requires prior ordering of the *purA* alleles with respect to *ampA*.

This indicates that the *purA* marker of ES4 lies to the right of both *purA28* and *purA31*. The failure to observe crossovers between the *purA* of ES4 and *hfl-1* therefore would seem to be due to some kind of crossover suppression phenomenon, rather than an extremely close proximity of these two loci.

Properties of hfl$^+$/hfl *merodiploids:* Having established that the *purA*-linked *hfl* mutants lay outside of and to the left of the *purA* locus, we next wished to establish whether *hfl* was dominant or recessive to *hfl*$^+$ in merodiploids. The F118 F-prime factor described by Low (1973) spans a 5-minute region of the *E coli* chromosome from *pyrB* to *malB*, including the *purA*, *hfl* and *ampA* loci, and is ideal for this purpose.

Merodiploids of the form F118 *hfl*$^+$ *pur*$^+$/*hfl purA* were constructed by mating F118/KL132 with *hfl purA* strains (Table 2, Column 2) or with *recA* derivatives of these strains. Merodiploids of the form F118 *hfl pur*$^+$/*hfl*$^+$ *purA* were

TABLE 6

Ordering of hfl *markers with respect to* ampA *and* purA

Strain	Genotype*	Donor or recipient	Case 1 or 2 (Figure 1)	Selected marker	Unselected markers		
					Hfl	Amp	Number
					—	+	29
UC3058	*ampA hfl-1 purA23*	D	1	Pur+	—	—	9
UC3031	*amp+ hfl+ purA19*	R			+	+	10
					+	—	0
					—	+	32
UC3061	*ampA hfl-59 purA29*	D	1	Pur+	—	—	6
UC3013	*amp+ hfl+ purA1*	R			+	+	8
					+	—	0
					—	+	24
UC3063	*ampA hfl-66 purA33*	D	1	Pur+	—	—	9
UC3031	*amp+ hfl+ purA19*	R			+	+	12
					+	—	3
					+	+	23
UC3060	*ampA hfl-58 purA28*	D	2	Pur+	+	—	4
UC3031	*amp+ hfl+ purA19*	R			—	+	12
					—	—	0
					+	+	29
UC3062	*ampA hfl-65 purA31*	D	2	Pur+	+	—	4
UC3031	*amp+ hfl+ purA19*	R			—	+	13
					—	—	2

* The order of the *purA* alleles was first determined by the method of MARGOLIN (1963). It is *ampA–(purA28, purA31)–purA19–purA1–(purA33, purA23)–purA29.*

TABLE 7

Ordering of purA *marker in ES4 with respect to* purA28 *and* purA31

Strain	Genotype	Donor or recipient	Selected marker	Unselected markers		
				Hfl	Amp	Number
				+	+	23
UC3060	*ampA hfl-58 purA28*	D	Pur+	+	—	2
ES4	*amp+ hfl+ purA45*	R		—	+	6
				—	—	1
				+	+	11
UC3062	*ampA hfl-65 purA31*	D	Pur+	+	—	0
ES4	*amp+ hfl+ purA45*	R		—	+	5
				—	—	0

constructed as described in MATERIALS AND METHODS (Table 3, Method 2). All the F′ *hfl*$^+$/*hfl* and the F′ *hfl*/*hfl*$^+$ merodiploids tested had an Hfl$^+$ phenotype. These included F118 *hfl*$^+$/*hfl recA* merozygotes with *hfl-1*, *hfl-58*, *hfl-65* and *hfl-66*, F118 *hfl*$^+$/*hfl recA*$^+$ merozygotes with *hfl-53* and *hfl-59*, and F118 *hfl*/*hfl*$^+$ *recA*$^+$ merozygotes with *hfl-53* and *hfl-58*.

The genetic structures of these merozygotes were verified in several ways: The structures of the F118 *hfl*$^+$ *pur*$^+$/*hfl purA recA* merozygotes were substantiated by their ability to transfer the Pyr$^+$ marker to the *pyrB* strain AT2535 and by their Pur$^+$ phenotype. Alternate structures are unlikely since the *recA* mutation reduces recombination between episome and chromosome to a negligible level (Low 1968). Similar observations were made with the F118 *hfl*$^+$ *pur*$^+$/*hfl purA recA*$^+$ merozygotes. Although we cannot be quite so sure that a rare recombinational event between chromosome and episome did not occur in these *recA*$^+$ strains to form an F118 *hfl*$^+$/*hfl*$^+$ recombinant, we do know that when we looked for such recombinants in constructing an F118 *purA*/*purA recA*$^+$ derivative (Table 1 and MATERIALS AND METHODS), they were quite rare. The structures of the F118 *hfl purA*$^+$/*hfl*$^+$ *purA recA*$^+$ merozygotes were substantiated by showing that bacteria purified from the plate on which the Hfl$^+$ phenotype was determined were still Pur$^+$ and were still able to transfer this Pur$^+$ characteristic

TABLE 8

Construction and complementation properties of F118 hfl-*x*/hfl-*y* recA *merodiploids*

Episome*	Chromosome					
	hfl-1	*hfl-53*	*hfl-58*	*hfl-59*	*hfl-65*	*hfl-66*
hfl-1	—†	—	—	—	+‡§	—
hfl-53	—	—	—	n.t.¶	—	—
hfl-58	n.t.	+	—	n.t.	+	—
hfl-59	±**	—	—	—	—	—
hfl-65	±	—	—	—	+††	—
hfl-66	+§	—	—‡‡	—	—‡‡	—

* Merodiploids with *hfl-1*, *hfl-59*, *hfl-65* and *hfl-66* on the episome were constructed by mating the appropriate merodiploids in Table 3 with F$^-$ *amp*$^+$ *hfl*-y *trp*$^+$ *recA* derivatives of strains in Table 2 Column 1 and selecting for Amp$^-$ Trp$^+$. Merodiploids with *hfl-53* and *hfl-58* on the episome were constructed by mating the appropriate merodiploids in Table 3 with F$^-$ *hfl*-y *purA trp*$^+$ *recA* derivatives of strains in Table 2 Column 2 (except the F$^-$ *hfl-53* recipient, which was strain UC3414) and selecting for Pur$^+$ Trp$^+$.

† — = Turbid λcIII plaques (Hfl$^-$ phenotype).

‡ + = Clear λcIII plaques (Hfl$^+$ phenotype).

§ This Hfl$^+$ F′ *hfl*-x/*hfl*-y *recA* merodiploid was mated with an F$^-$ *hfl-66 recA* strain to obtain a F′ *hfl*-x/*hfl-66 recA* merodiploid. The new merodiploid had an Hfl$^-$ phenotype, as would be expected from examination of the Table.

¶ n.t. = Not tested.

** ± = Slightly turbid λcIII plaques.

†† This Hfl$^+$ F′ *hfl-65*/*hfl-65 recA* merodiploid was mated with an F$^-$ *hfl-66 recA* strain to obtain a F′ *hfl-65*/*hfl-66 recA* merodiploid. The new merodiploid, which had the expected Hfl$^-$ phenotype, was then mated with an F$^-$ *hfl-65 recA* merodiploid to obtain a F′ *hfl-65*/*hfl-65 recA* merodiploid once more. Like the original merodiploid, the new merodiploid had an Hfl$^+$ phenotype.

‡‡ This Hfl$^-$ F′ *hfl-66*/*hfl*-y *recA* merodiploid was mated with an F$^-$ *hfl-1 recA* strain to obtain a F′ *hfl-66*/*hfl-1 recA* merodiploid. The new merodiploid had an Hfl$^-$ phenotype, as would be expected from examination of the Table.

to an F⁻ *purA* strain. The presence of the *hfl* marker on the episomes of these merozygotes was demonstrated by their successful use as donors in producing the merozygotes in lines 2 and 3 of Table 8.

We can, therefore, conclude with some confidence that all six *hfl* mutants are recessive to *hfl*⁺. This conclusion was strengthened by the subsequent finding that all six mutants lay in a single complementation group.

For complementation studies, strains carrying different *hfl* mutations on the F118 episome were constructed as described in MATERIALS AND METHODS (Table 3). These strains were then mated with *recA* derivatives of F⁻ *hfl* strains to form *recA* merodiploids of the form F118 *hfl*-x/*hfl*-y (Table 8). Most combinations resulted in an Hfl⁻ phenotype (Table 8), indicating that all six hfl mutants lie in a single complementation group. There were a few examples of apparent intragenic complementation, notably F118 *hfl-58/hfl-53*, F118 *hfl-66/hfl-1*, and, to a marginal extent, F118 *hfl-59/hfl-1* (Table 8). In all three cases, the reciprocal pairs did *not* complement, which is possibly due to a gene dosage effect stemming from multiple copies of episome per chromosome. The mutation *hfl-65* has only a marginal *hfl* phenotype in quantitative lysogenization tests and it should not be surprising that merodiploids containing the *hfl-65* mutation frequently show apparent complementation. The fact that *hfl-65* apparently "complements" itself could be understood in terms of a marginally stable protein synthesized in greater quantities in an F118 *hfl-65/hfl-65* merodiploid than in the corresponding F⁻ *hfl-65* haploid strain.

Intragenic recombination between hfl *mutants:* Since all six *purA*-linked *hfl* mutants lie in a single complementation group, we did not attempt to order the different *hfl* mutants. We did show that intragenic recombination occurs, which demonstrates that the *hfl* mutants occur at different sites on the chromosome. For example, when UC3061 (*ampA hfl-59 purA29*) was the donor and UC3035 (*ampA*⁺ *hfl-1 purA23*) the recipient, 88 Pur⁺ recombinants were Hfl⁻ like the parents and 22 Pur⁺ recombinants were Hfl⁺ recombinants as well. The 88 Hfl⁻ Pur⁺ recombinants included 71 Amp⁺ and 17 Amp⁻ colonies. All 22 Hfl⁺ Pur⁺ double recombinants were Amp⁺, which indicates an order of *ampA–hfl-59–hfl-1–purA*. The reciprocal cross was not done because we did not have suitably marked *purA* derivatives and crosses with a *pur*⁺ donor gave considerably lower frequencies of recombination between *hfl* mutants. This latter observation, as well as the lower frequencies of recombination shown in Table 4 than in Table 6, is an example of the well known phenomenon of high negative interference between closely linked markers (CHASE and DOERMANN 1958).

DISCUSSION

These results show that six *Escherichia coli* K12 *hfl* mutants lie in a single complementation group which is tightly linked to the *purA* locus, but counterclockwise and separable from it. All six mutants have the same phenotypic properties: (1) Lambda λ*cIII* mutants form turbid plaques on these mutants. (2) Lambda wild type and λ*cIII* mutants lysogenize these mutants more efficiently

than *hfl*$^+$ strains. (3) Lambda wild type, λ*cIII* and λ*c17* show a reduced plaque-forming ability on these mutants. (4) These mutants all show a slightly increased resistance to penicillin and rifamycin. (5) These mutants are all slightly more sensitive to sodium dodecyl sulfate. These similar phenotype properties, the recessive nature of *hfl* mutations to *hfl*$^+$ in merodiploids, and the complementation behavior of *hfl* mutants suggest that the *hfl* mutation represents a *loss* of function and that the *hfl*$^+$ gene is a classical gene, probably coding for the synthesis of a protein. We propose to call the *purA*-linked *hfl* locus the *hflA* locus.

An estimate of the distance between the *hflA* and *purA* loci may be obtained from the formula of Wu (1966), relating co-transduction frequencies to map distance, which is being used in construction of the *E. coli* K12 genetic map (Taylor and Trotter 1972). From Table 4 it may be calculated that the average co-transduction frequency between *hflA* and *purA* mutants is 92%, from which a 0.054 minute separation between the *hflA* and *purA* loci may be calculated. Assuming 5000 genes in the *E. coli* chromosome and a total map length of 90 minutes, this would mean that the *hflA* and *purA* loci were only 3 genes apart, which is to say that there were only 2 intervening genes. Although calculations based on co-transduction frequencies are necessarily imprecise, they do emphasize the close proximity of the *hflA* and *purA* loci. It is possible that the two genes are adjacent to one another. Thirteen *purA* mutants of UC4185 isolated in this study did not revert to wild type and may be deletions. However, all of these mutants had an Hfl$^+$ phenotype and all recombined with both *purA28* and *purA29*, which are at the left and right ends of our *purA* fine structure map (Table 6). Thus, while it is conceivable that non-lethal deletions may extend from the *purA* locus into the *hflA* locus, we did not find any.

Our finding of isolated instances of intragenic complementation in F118 *hfl*-x/*hfl*-y merodiploids fortifies our conclusion that *hflA* locus is a classical gene. Indeed, all instances of intragenic complementation deal with classical gene products, namely enzymes (Schlesinger and Levinthal 1965; Fincham 1966). Intragenic complementation is known to involve interactions of subunits in multimeric proteins. However, care must be taken in interpreting the data on the *hflA* locus because in no instance was intragenic complementation reciprocal. This could be caused by unequal amounts of synthesis of the *hflA* product from episomal and chromosomal genes.

If the *hfl*$^+$ gene indeed determines the synthesis of an Hfl$^+$ protein, then how are the high frequencies of lysogenization of *hflA* strains by λ$^+$ phage and λ*cIII* mutants to be interpreted? The obvious interpretation is that the Hfl$^+$ protein normally antagonizes lysogenization, and that the product of the λ*cIII* gene negates this antagonistic effect, although its mode of action could be indirect. The idea of a phage protein acting to inhibit a host protein is not new. Roberts (1969) has suggested that the N protein of λ inhibits the host termination factor, rho, allowing transcription to continue past normal termination signals.

The λ*cIII* gene product promotes transcription for repressor synthesis at a promoter called *pre*, the promotor for repressor establishment (Reichardt and Kaiser 1971; Echols and Green 1971), which would imply that efficient tran-

scription from this promoter must occur in the absence of the lambda *cIII* gene product in an *hflA* host. How could the Hfl$^+$ protein antagonize transcription? One possibility is that it controls the concentration of some small molecule which interacts at the promoter with RNA polymerase to control transcription. This small molecule could be a nucleotide, but could not be adenosine 3′:5′-cyclic monophosphate (BELFORT and WULFF 1973). A second possibility is that the Hfl$^+$ protein is normally bound to RNA polymerase but is not essential for its activity. (A number of such proteins may exist (TRAVERS and BUCKLAND 1973).) Such a polymerase molecule would be hypothesized to be unable to transcribe from the *pre* promoter unless λcIII protein were present to negate somehow the inhibitory effect of the Hfl$^+$ protein.

It will be of great interest to identify the function of the Hfl$^+$ protein. While the existence of the *hflA* mutants implies that the Hfl$^+$ protein is not an essential host protein, it is possible that all *hflA* mutants have at least partial Hfl$^+$ activity and that some Hfl$^+$ activity is required for the host cell to function. Indeed, all six of the *hflA* mutants retain their Hfl$^-$ phenotypes when transduced into the *su*$^+$ strain ES4, which suggests that none of them are amber mutants. The fact that the percent lysogenization of the six *hflA* mutants by a λ*cIII* phage strain varies over a wide range indicates that most or all *hflA* mutants have at least partial Hfl$^+$ function. While this might mean that a totally inactive Hfl$^+$ protein is lethal to the host, it also could merely be a consequence of most NG-induced mutants having partial activity, combined with a very sensitive test for detecting *hflA* mutants.

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LITERATURE CITED

BELFORT, M. and D. L. WULFF, 1971 A mutant of *Escherichia coli* that is lysogenized with high frequency. pp. 739–742. In: *The Bacteriophage Lambda.* Edited by A. D. HERSHEY. Cold Spring Harbor, New York. ——, 1973a A genetic and biochemical investigation of the *Escherichia coli* mutant *hfl*-1 which is lysogenized at high frequency by bacteriophage lambda. J. Bacteriol. **115:** 299–306. ——, 1973b An analysis of the processes of infection and induction of *Escherichia coli* mutant *hfl*-1 by bacteriophage lambda. Virology **55:** 183–192.

CHASE, M. and A. H. DOERMANN, 1958 High negative interference over short segments of the genetic structure of bacteriophage T4. Genetics **43:** 332–353.

ECHOLS, H. and L. GREEN, 1971 Establishment and maintenance of repression by bacteriophage lambda: The role of the cI, cII, and cIII proteins. Proc. Natl. Acad. Sci. U.S. **68:** 2190–2194.

ERIKSSON-GRENNBERG, K. G., 1968 Resistance of *Escherichia coli* to penicillins. II. An improved mapping of the *ampA* gene. Genet. Res. **12:** 147–156.

FINCHAM, J. R. S., 1966 *Genetic Complementation.* W. A. Benjamin Press, New York.

GOTTESMAN, M. and M. YARMOLINSKY, 1968 Integration negative mutants of lambda. J. Molec. Biol. **31:** 487–505.

KEMPER, J. and P. MARGOLIN, 1969 Suppression by gene substitution for the *leuD* gene of *Salmonella typhimurium.* Genetics **63:** 263–279.

LIEBERMAN, I., 1956 Enzymatic synthesis of adenosine-5′-phosphate from inosine-5′-phosphate. J. Biol. Chem. **223:** 327–339.

Low, B., 1968 Formation of merodiploids in matings with a class of Rec⁻ recipient strains of *Escherichia coli* K12. Proc. Natl. Acad. Sci. U.S. **60**: 160–167. ——, 1973 Rapid mapping of conditional and auxotrophic mutations in *Escherichia coli* K12. J. Bacteriol. **113**: 798–812.

Luria, S. E., J. N. Adams and R. C. Ting, 1960 Transduction of lactose utilizing ability among strains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. Virology **12**: 348–390.

Margolin, P., 1963 Genetic fine structure of the leucine operon in Salmonella. Genetics **48**: 441–457.

Mount, D. W., K. B. Low and S. J. Edmiston, 1972 Dominant mutations (*lex*) in *Escherichia coli* K-12 which effect radiation sensitivity and frequency of ultraviolet light-induced mutations. J. Bacteriol. **112**: 886–893.

Reichardt, L. and A. D. Kaiser, 1971 Control of lambda repressor synthesis. Proc. Natl. Acad. Sci. U.S. **68**: 2185–2189.

Roberts, J. W., 1969 Termination factor for RNA synthesis. Nature **224**: 1168–1174.

Schlesinger, M. J. and C. Levinthal, 1965 Complementation at the molecular level of enzyme interactions. Ann. Rev. Microbiol. **19**: 267–284.

Signer, E. R., K. F. Manly and M. Brunstetter, 1969 Deletion mapping of the cIII–N region of bacteriophage lambda. Virology **39**: 137–141.

Stouthamer, A. H., P. G. de Haan and H. J. J. Nijkamp, 1965 Mapping of purine markers in *Escherichia coli* K12. Genet. Res. **6**: 442–453.

Taylor, A. L. and C. D. Trotter, 1972 Linkage map of *Escherichia coli* strain K12. Bacteriol. Rev. **36**: 504–524.

Travers, A. and R. Buckland, 1973 Heterogeneity of *E. coli* RNA polymerase. Nature New Biol. **243**: 257–260.

Vogel, H. J. and D. M. Bonner, 1956 Acetyl-ornithase of *Escherichia coli*: Partial purification and some properties. J. Biol. Chem. **218**: 97–106.

Wu, T. T., 1966 A model for three-point analysis of random general transduction. Genetics **54**: 405–410.

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