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Extent of Host Deletions Associated with Bacteriophage P2-Mediated Eduction

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A series of independent *Escherichia coli* K eductants has been isolated and tested to determine the extent of their deletions. The deletions cover the P2 prophage in location H, the *his* operon, a suppressor of the *recBC* phenotype (*sbcB*), the gene for gluconate-6-phosphate dehydrogenase (*gnd*), a locus involved in cell wall synthesis (*rfb*), and in some cases all or part of genes involved in methylgalactoside uptake (*mglP*). One end of the deletion, the P2 prophage end, appears to be the same for all eductants. The other end, however, can be located before, within, and after the *mglP* locus.

The temperate bacteriophage P2 can establish itself as prophage at several different sites on the chromosomes of *Escherichia coli* strains C and K-12 (7, 22, 32). P2 conforms to the Campbell model when it establishes lysogeny (11) and requires P2*int* product for integration and excision (13, 26).

Protrophic *E. coli* K-12 strains (hereafter called K) lysogenic for phage P2 in location H, closely linked to the histidine locus, segregate histidine-requiring cells (23). This phenomenon is termed "eduction" (eduction may be applied to all cases of a loss of host genetic material concomitant to the exit of an integrated episome), and the deletion-containing strains are called eductants. Eductants are thought to contain deletions extending through the *his* operon into adjacent genes, since they do not revert to his^+ and grow more slowly than *E. coli* K his^- point mutants (23). In the present paper we report the isolation of a series of independent eductants and studies on the nature and extent of the deletions. In addition, the position of P2 location H has been mapped with reference to several host markers.

MATERIALS AND METHODS

Bacteria. *Escherichia coli* K-12 and C strains and their derivatives listed in Table 1 were used. The *Salmonella typhimurium* strains used in the F'his^- crosses (not listed in Table 1) contained F'his^- episomes of *E. coli* K-12 origin and were generously supplied by P. Hartman. The histidine mutations on the F'*his* episomes were identified by Garrick-Silversmith and Hartman (19) as to the gene locus involved. These included genes *I*, *F*, *A*, *B*, *C*, *D*, a small deletion *BC*, and a large deletion *EIFAH*. A series of *E. coli* K-12 strains carrying known mutations in various genes of the histidine operon were obtained from P. Hartman and used to test the *Salmonella* strains which carry F'*his*. C-8, C-18, C-520, and C-1055 were used as indicators for P2. All incubations were performed at 37 C unless otherwise indicated.

Phage. Phage strains used are listed in Table 2. General phage techniques used were those described by Adams (1).

Media. The media used, as well as the methods employed with phage P2, have been described by Bertani (3, 4), Bertani and Six (7), and Kelly and Sunshine (23). The media include: NB, nutrient broth (Difco) supplemented with 0.5% NaCl; LB, a richer broth, SA, LA, and HLA, solid media prepared from LB by the addition of 0.7, 1, or 1.5% agar, respectively; MacConkey-gluconate indicator plates (29) containing 4% MacConkey agar base (Difco), to which 1% sodium gluconate was added; and Davis minimal agar (DMA, formula in reference 31). When amino acids were added to DMA, they were used at a final concentration of 20 μg of the L-form per ml for normal growth. For detection of eductants, L-histidine was added at a concentration of 0.5, 1.0, or 2.0 μg/ml (low *his* plates) depending upon the bacterial strain to be tested.

Transduction experiments. Transductions were performed as previously described (35). In the case of gnd^+-selected transductants, the methods of Peyru and Fraenkel (29) were followed. Colonies were streaked for reisolation on MacConkey-gluconate plates. In this way, gnd^+ transductants could be differentiated by the color of the colonies from edd^+ transductants since the original selective plates supported the growth of both types. Reisolated colonies were picked to LB in autoclavable plastic chambers containing 25 wells and incubated overnight. Unselected markers were tested by

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TABLE 1. *Bacterial strains used*

Strain no.	Pertinent genetic structure[a]	Source or origin
AB311	Hfr *thi leu thr lac-4 str*^{r}	M. B. Rotman
AB1360	F$^-$ *his pro arg aroD thi*	(30)
AB1360	AB1360 *his*$^+$	Spontaneous *his*$^+$ revertant of AB1360
AB2463	*recA* polyauxotrophic	E. A. Adelberg
AB2880	AB1360 *shiA*	(30)
AT2572	Hfr, *his* is an early maker	E. A. Adelberg
AT2572 (P2 *rd l c*)$_1$	(P2 *rd l c*)$_H$	From AT2572 by lysogenization
C-8	F$^-$ *trp arg str*^{r}	(7)
C-18	F$^-$ (λ)	(7)
C-520	F$^+$ *supD*	(36)
C-1055	F$^-$ polyauxotrophic	(39)
CR-63	*supD*	(38)
DF-412	HfrC *edd gnd his thi*	(29)
HfrC-1	HfrC	(15)
HfrC-2	HfrC *glpT*	(15)
K-12	F$^+$ (λ) *glpT*	G. Bertani
K-40	HfrH *thi*	G. Bertani
LG-4	K-12 (P2)$_H$	K-12 (P2) isol. 4 of Kelly (22)
LG-5	K-12 (P2)$_H$	K-12 (P2) isol. 5 of Kelly (22)
LG-103	K-40 (P2)$_H$	K-40 (P2) isol. 3 of Kelly (22)
LG-106	K-40 (P2 lg)$_H$	K-40 (P2) isol. 6 of Kelly (22)
LG-110	LG-106 *his*	Reverting *his* mutation found by UV[b] irradiation and penicillin screening
LG-111	K-40 *his*	Reverting *his* mutation found by UV[b] irradiation and penicillin screening
LG-117 LG-118 LG-119 LG-121	HfrH *thi* (P2 *rd l c*)$_H$	(23)
LG-122	HfrH *thi* (P2 *rd l c*)$_H$ (P2 *rd l c*)$_X$	(23)
LG-128	*recA his*$^+$	Spontaneous *his*$^+$ revertant of AB2463
LG-129	*recA* (P2)$_H$	From LG-128 by lysogenization
LG-202	*his, trp, met, str*^{r} (P2)$_H$ (λ)	K-43 (P2) isol. 3 of Kelly (22)

[a] Symbols: F$^-$, F$^+$, Hfr—fertility types; (P2)$_{H,X}$—carrying prophage P2 in location H or unknown location; λ—lysogenic for λ; *trp, arg, his, pro, met, pyrD, thi*—requiring tryptophan, arginine, histidine, proline, methionine, pyrimidine, thiamine, respectively; *glpT*—mutated in α-glycerol phosphate transport; *edd*—mutated in gluconate-6-phosphate dehydrase; *gnd*—mutated in gluconate-6-phosphate dehydrogenase; *aroD*—requiring tyrosine, tryptophan, and phenylalanine; *shiA*—mutated in shikimic acid permease; *recA*—recombinationless; *supD*—carrying suppressor D; *str*^{r}—streptomycin resistant; *lac-4*—unable to ferment lactose.

[b] Ultraviolet.

replicating with a multiprong replicator onto appropriately supplemented DMA plates for amino acid markers and to LA plates seeded with an indicator strain to test for the presence of phage.

Bacterial crosses. Uninterrupted mating crosses were performed as previously described (35). For interrupted mating crosses, log-phase Hfr and F$^-$ cells grown without shaking in NB plus 0.1% glucose were mixed in fresh broth at a ratio of 1:20 and shaken gently at 37 C. After 5 min, the mixture was gently diluted 1:50 into prewarmed NB plus 0.1% glucose. At intervals, 1-ml samples were removed, vigorously agitated for 1 min on a Vari-Whirl mixer, and diluted, if necessary, in the same medium. One-tenth-milliliter amounts were plated on appropriately supplemented Davis agar in a 2.5 ml of soft agar overlayer.

Production and selection of eductants. Single colony isolates from *his*$^+$ *E. coli* K strains carrying P2 prophage in location H were picked to NB or LB, incubated overnight, diluted 10^6-fold, and spread on low *his* plates. After 48 hr of incubation, such plates usually produced 300 to 500 large, opaque colonies and several small, translucent colonies. Small, translucent colonies were also isolated by picking the bacterial growth from the centers of plaques of P2 on sensitive K strains and then treating as above. The small, translucent colonies were picked to LB, grown overnight, and spotted on DMA plates with and without histidine to ascertain that they were *his*$^-$, and LA indicator plates to test for the production of phage. Histidine-requiring, nonlyso-

genic strains arising from P2 lysogens and *his*⁻ strains produced from P2 infection experiment were saved for further testing.

Testing of eductants. Eductants were reisolated and subjected to the following tests.

Phage production and sensitivity. Eductants were tested for the ability to produce P2 by spotting on susceptible indicator strains and for sensitivity to P2, P2*vir3*, P1, ϕX174, and a series of temperate phage from the collection of G. Bertani, by cross streaking against phage from stocks containing approximately 2 $\times$ 10^8 plaque-forming units (PFU) per ml.

Reversion to histidine independence. Strains were tested for spontaneous reversion to histidine independence and for reversion caused by three mutagens: diethyl sulfate (DES, Matheson Coleman and Bell), *N*-methyl-*N'*-nitro-nitrosoguanidine (NG, Aldrich Chemical Co.), and 3-chloro-7-methoxy-9 (3-chlorethyl amino-propylamine) acridine dichloride (ICR-191, gift of Hugo J. Creech, Institute for Cancer Research, Philadelphia, Pa.). Bacteria were grown in LB overnight, and samples containing 4 $\times$ 10^8 to 8 $\times$ 10^8 bacteria were plated in soft DMA (0.7%) on properly supplemented DMA plates containing 0.01% NB. One drop of sterile 1% NaCl or the appropriate mutagen (DES, one drop; NG, one drop of freshly prepared 2-mg solution per ml; ICR, one drop of a 0.1-mg solution per ml) was placed on a sterile, filter-paper disc in the center of the plate. Plates were incubated for 2 to 3 days and inspected for the presence of prototrophic colonies. A known reverting mutant was always included as control.

Genetic mapping by F'his⁻-mediated conjugation. Genetic mapping of histidine-requiring mutants was performed by using a set of *S. typhimurium* strains carrying F'*his* episomes of *E. coli* K-12 origin. An F'*his*⁺ donor was always included as control. The spot test crossing technique is modified from Garrick-Silversmith and Hartman (19). Control crosses by using known *E. coli* K *his*⁻ strains and two eductants as recipients were performed to standardize the procedure.

Bacteria were grown overnight in NB plus 0.1% glucose. DMA plates supplemented with thiamine and amino acids as needed were spread with 0.1-ml amounts of the eductants (recipients) and then spotted with drops of the F'*his* donor strains (nine donors per plate). Controls of donor strains alone and donor strains spotted on recipients with known *his*⁻ mutations were performed at the same time. Plates were incubated for 2 days at 37 C, examined for prototrophic growth, left at room temperature for 1 day, and again examined for slow growing prototrophs. When two strains in combination produced no growth, it was inferred that the two were defective for at least one gene in common.

Garrick-Silversmith and Hartman (19) reported that both recombination and complementation were involved in prototroph production when *Salmonella* strains carrying *E. coli* F'*his* elements were mated with *E. coli* mutants. We found, however, that when the *E. coli* strain also carried the *recA* mutation, no prototrophs were produced. This indicates that recombination plays a major role in the production of prototrophs.

The spot tests usually give clear-cut, all-or-none results when applied to deletion-containing strains. When used to identify point mutations, the number of cells used is critical and the test is sometimes ambiguous.

TABLE 2. *Bacteriophage strains used*

Strain	Source or reference
P1*kc*	25
P2	4
P2*lg cc*	6
P2*xt*	4
P2*hy dis*	14
P2*vir1*	9
P2*vir3*	8
P2*vir3 am201*	36
P2*am208*	36
P2*vir3 ts4*	26
P2*vir3 ts13*	26
P2*vir24 ts40*	26
λPaPa	16
ϕD series	G. Bertani
ϕX174	G. Bertani

Prophage marker rescue experiments. Samples (0.2 ml) of temperature-sensitive (*ts*) P2*vir* (immunity insensitive) stocks containing 0.7 $\times$ 10^8 to 2.4 $\times$ 10^8 phage per ml were plated with selected eductants and incubated at 42 C, at which temperature the P2*ts vir* phage cannot grow. *E. coli* K-40 was included as control to determine the presence of non-temperature-sensitive revertants in phage stocks. *E. coli* LG-106 was used as control to check that the presence of a wild-type P2 prophage would allow rescue of the temperature-sensitive phage at the nonpermissive temperature. In this way, the temperature-sensitive phage would form plaques at 42 C through complementation or recombination with the corresponding *ts*⁺ allele if one were present in the eductants (Table 9).

Interference with phage lambda. *E. coli* K strains lysogenic for phage P2 do not plate phage λ (5, 27). To test whether eductants would support the growth of λ, 10^6 λPaPa was spotted on lawns of eductants, or 10^3 phage were plated for single plaques (Table 9).

Gluconic-6-phosphate dehydrogenase (gnd). Tests for the presence of *gnd*⁺ were performed by using the color assay described by Peyru and Fraenkel (29). In addition, a small series of eductants was tested for *gnd* enzymatic activity by H. Nikaido (Table 7).

Thymidine diphosphorhamnose genes (rfb). A small series of eductants was tested by H. Nikaido for enzymatic activities of *rha-1*, *rha-2*, and *rha-3* genes which are part of a locus governing the biosynthesis of nucleotide sugars and which, most likely, correspond to the *rfb* locus in *S. typhimurium* (Table 7).

L-α-Glycerol phosphate transport. Screening of strains for the presence of *glpT* was performed by spotting on DMA supplemented with 0.2% L-α-glycerol phosphate. Strains which are *glpT*⁺ can grow with L-α-glycerol phosphate as the sole carbon source (15).

Shikimic A (shiA). Eductants from *shiA*⁺ *his*⁺ *aroD*⁻ $(P2)_H$ strains were selected by plating on low *his* agar supplemented with tyrosine, tryptophan, and phenylalanine and then tested for ability to grow when shikimic acid was substituted for these three amino acids (30).

Motility. Eductants were tested for motility by stabbing tubes of soft nutrient agar (0.4% agar) plus 2,3,5-triphenyl-tetrazolium chloride (0.001%) and by spotting on sloppy agar plates (33). Tubes and plates were checked for motility after 1, 2, and 3 days.

UV sensitivity (uvrC). Selected eductants were examined for the *uvrC* marker by testing their ability to repair ultraviolet (UV)-irradiated λ. Bacteriophage λ was irradiated for 60 sec at a dose of 80.8 ergs per mm^2 per sec and plated on strains to be tested. Plating was done in the presence of subdued yellow light and plates were incubated in the dark (Table 8).

Suppressor D (supD). Eductants arising from strains carrying *supD* were tested for the presence of the suppressor by cross streaking against lysates of P2*am201 vir3* and P2*am208* containing 5×10^7 PFU/ml.

Resistance to high levels of nalidixic acid (nalA). Eductants were tested for the presence of the *nalA* gene by selecting for mutants resistant to high levels (40 μg /ml) of nalidixic acid by the method of Hane and Wood (21). In addition, eductants from a *nalA* derivative of LG-106 were tested to determine whether they still showed the same level of resistance to nalidixic acid as the parental strain.

Methylgalactoside permease (mglP). R. Guzman and J. Dietrich measured the methyl-β-D-galactoside permease activity of the eductants by the intracellular accumulation of D[*methyl*-β-^{14}*C*]galactoside by the procedure of Ganesan and Rotman (18) (Table 10).

Suitable control strains giving positive and negative results in the above tests were always included.

RESULTS

Mapping of P2 location H with respect to the his and gnd loci. Bacterial conjugation and transduction experiments (22, 35) have shown a close linkage of P2 prophage location H with the histidine locus but did not give any indication as to the order of these markers in reference to other bacterial markers. This sequence was determined by interrupted mating crosses by using as donor AT2572, an *Hfr* strain which injects in the order *O-his-trp-thr* with *his* as an early marker and LG-202 as recipient. Since the percentage of his^+-selected recombinants carrying the donor $(-)_H$ increased with time of sampling, the results suggested the order *-his-*$(P2)_H$*-trp-*(Table 4). To confirm this order, P1 transductions were performed by using LG-106 as donor and DF-412 as recipient and selections were made separately for his^+ and gnd^+ transductants. The sequence of these two markers with respect to *trp* is *gnd-his-trp* (29, 37). The data are presented in Table 5.

The three markers *his*, *gnd*, and $(P2)_H$ were cotransducible. However, with selection for gnd^+, the observed cotransduction frequencies for the two unselected markers differed from the values expected for independence of transfer, and the least frequent transduction class, his^- $(P2)_H$, interpreted as the quadruple exchange class, indicates the sequence *gnd-his-*$(P2)_H$. With selection for his^+, the observed cotransduction frequencies for the unselected markers were as expected if their transfers were independent. This is compatible with the unselected markers being on opposite sides of *his*. The data best fit the sequence *gnd-his-*$(P2)_H$ and thus confirm the sequence *his-*$(P2)_H$*-trp* suggested from the interrupted mating crosses.

Mapping of P2 location H with respect to the his and shiA loci. Pittard and Wallace (30) have determined the order *his-shiA-trp*. P1 transductions were performed with *LG*-106 (his^+, $shiA^+$, lysogenic for P2) as donor and AB2880 (his^-, $shiA^-$, $aroD^-$) as recipient to determine where P2 location H is in relation to *his* and *shiA*. With selection for his^+, 47% cotransduction of P2 and 12% cotransduction of $shiA^+$ were obtained. Twenty-three per cent of the his^+ transductants carrying P2 were also $shiA^+$, whereas 92% of the his^+ transductants carrying $shiA^+$ were also lysogenic for P2. With selection for his^+ $shiA^+$, 97% of transductants were lysogenic for P2. Selection for $shiA^+$ transductants was unsuccessful since only 1.7% (5/300) of the colonies tested were $shiA^+$; the remainder were $aroD^+$. Pittard and Wallace (30) have reported that approximately 50% of the transductants upon selection for $shiA^+$ are $shiA^+$, the remainder being $aroD^+$. The unexplained result obtained here has not been investigated further. The data indicate the order *his-*$(P2)_H$*-shiA*. Calendar and Lindahl (11) have shown that in his^+ transductions with donor and recipient strains with differently marked location H prophages, the majority of transductants receiving the $shiA^+$ locus from the donor also received the whole donor prophage. These data confirm the order *his-*$(P2)_H$*-shiA*.

Extent of the deletions. In a previous report, Kelly and Sunshine (23) have shown that a small series of histidine-requiring eductants did not revert to histidine prototrophy either spontaneously or in the presence of chemical mutagens. This indicated that the eductants contain deletions affecting the histidine operon. Similarly, in the work reported here 132 eductants, 81 of known independent origin obtained from several location H lysogens and from P2 infection experiments (Table 3), were tested for reversion to histidine prototrophy. They were found not to revert either spontaneously or in the presence of chemical mutagens.

To test for the extent of the deletions within the *his* operon, 77 eductants (QE 1-74 and QE 130-132) were crossed with F'his^--carrying strains with separate mutations in six of the nine genes of the histidine operon. When two strains in combination produced no growth, it was in-

TABLE 3. *List of eductants*

Strain no.	Derivation	Origin
QE1-QE6	LG-106	Same event[a]
QE7-QE34	LG-106	Independent events
QE35-QE36	LG-103	Independent events
QE37-QE42, QE45-QE46	K-40 (P2 *rd l c*)$_H$	Independent events from each of 4 separate lysogenic strains
QE43-QE44	K-40 (P2 *rd l c*)$_H$(P2 *rd l c*)	2 Independent events
QE47-QE51	LG-4	Same event[a]
QE52-QE56	LG-5	Same event[a]
QE57-QE58	AT 2572 (P2 *rd l c*)$_1$	Independent events
QE61-QE62	P2 + K-40[b]	Independent events
QE63-QE64	P2 *hy dis* + K-40[b]	Independent events
QE65-QE66	P2 *xt* + K-40[b]	Independent events
QE67-QE68	P2 + K-12[b]	Independent events
QE69-QE71	P2 *hy dis* + K-12[b]	Independent events
QE72	P2 *xt* + K-12[b]	Independent event
QE73-QE74	P2 + CR3[b]	Independent events
QE75-QE94	P2 *lg* + AB311[b]	Independent events
QE95-QE129	P2 *lg cc* + AB1360 *his*$^{+b}$	10 Independent events
QE130-QE132	LG-129	Independent events

[a] Separate colonies isolated from the same culture. Possibly derived from the same eduction event.
[b] These eductants did not arise from known lysogenic strains but from infection experiments. Kelly and Sunshine (23) have shown that eductants can be isolated from plaques of P2 on sensitive K strains.

ferred that the two lacked at least one common function. The results summarized in Table 6 show that the six histidine genes tested are missing in all 74 eductants. Three eductants from a *recA* (QE 130-132) strain did not give recombinants with any of the F'*his* strains.

The finding that six of the nine histidine genes are missing in the eductants suggested that the whole histidine operon was deleted and that the deletions might extend into neighboring genes. This was strengthened by the observation that eductants grow more slowly than the parental strains and strains with *his*$^-$ point mutations both in NB and on DMA in the presence of excess histidine.

The chromosome of *E. coli* K-12 in the vicinity of the histidine operon is shown in Fig. 1 (18, 19, 24, 37). The approximate position of P2 location H based on the results in Tables 4 and 5 is also indicated.

To determine whether other genes were deleted, 77 eductants were tested for the presence or absence of some of the genes shown in Fig. 1, by using the procedures outlined above. The results are shown in Table 6, part I. In addition to *his* and (P2)$_H$, all of the 77 eductants tested were found to lack *gnd*.

The majority of the eductants were tested for *gnd* activity by using the color assay described by Peyru and Fraenkel (29). A small series of eductants was tested by H. Nikaido for enzyme activities corresponding to the gene *gnd*$^+$ and to three of the genes belonging to the *rfb* locus. The results shown in Table 7 corroborate the loss of *gnd* activity in the eductants. Since in *Salmonella* the genes governing those *rfb* enzymes tested are located distally in the *rfb* locus to *gnd* and *his* (28), it seems probable that the *rfb* enzymes not tested are also missing.

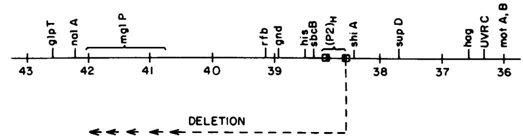

FIG. 1. *Genetic map of Escherichia coli K in the histidine region (18, 24, 37; Tables 4 and 5). Only the various bacterial markers tested are included. P2 location H is represented by an integrated prophage. The numbers represent divisions of the chromosome based on a total length of 90. Symbols: motA, B, motility; uvrC, repair of ultraviolet radiation damage to DNA; hag, flagellar antigens; supD, suppressor of amber mutations; shiA, shikimic acid permease; (P2)$_H$, in prophage in location H; his, histidine operon; sbcB, suppressor of recB, C phenotype; gnd, 6-phosphogluconic dehydrogenase; rfb, rough B; mglP, methyl-β-D-galactoside permease; nalA, resistance to high levels of nalidixic acid; glpT, α-glycerol phosphate transport.*

A suppressor of the *recB*, *C* phenotype, *sbcB*, is cotransducible with *his* (24). A. Templin and A. J. Clark (*personal communication*) have shown that all eductants arising from a *recB*$^-$ strain are *sbcB*$^-$. These results suggest that *sbcB* is deleted when eduction occurs.

Motility in *E. coli* is controlled by several

genes in this region (2) including *hag*, a structural gene for flagellin, located near *uvrC* (Fig. 1). Since the eductants were motile (Table 6), this region has not been deleted.

Eleven eductants were tested for the presence of an intact *uvrC* gene as shown in Table 8. All showed normal ability to repair irradiation damage in phage λ. Therefore, the *uvrC* gene is not deleted.

TABLE 4. *Transfer of P2 location H in interrupted mating experiments: AT-2572 × LG-202*

Expt	Time of sampling (min)	Selection for *str*ʳ and		
		his⁺		*trp*⁺
		Recombinants (% input Hfr)	Per cent nonlysogenic for P2[a]	Recombinants (% input Hfr)
1	10	0.46	54	0
	15	2.3	76	0
	20	4.0	88	0
	30	4.6	—[b]	0.17
2	8	0.2	51	—
	11	1.5	60	—
	15	2.7	83	—
	20	3.8	80	—
	25	5.0	82	—
	30	5.4	88	—

[a] Fifty colonies from each sample were tested after streaking once on the same selective medium for reisolation. In these crosses, the donor was nonlysogenic to eliminate the possibility of zygotic induction.

[b] Indicates not tested.

To determine whether the deletion extends through the *supD* locus, two eductants arising from P2 plaques on CR-63 were tested by cross streaking with P2 *amber* mutants. Both eductants were lysed by the *amber* mutants and therefore retained *supD*.

To determine whether *shiA* was deleted, eductants were isolated after P2 infection of an *aroD* strain. Tests with these eductants are recorded in Table 6, part II (QE-95-129); *shiA* was not deleted in the eductants tested.

The data in Table 6 show that as a general rule eductants do not produce phage P2 and are sensitive to infection by P2. These data do not eliminate the possibility of the presence in the eductants of a defective prophage similar to that in the cryptic lysogens of phage λ which do not produce phage or immunity substance (17). To test for the presence of defective P2 prophages, several eductants were screened for prophage marker rescue of conditional lethal P2 mutants in genes B, D, and H (26) and for λ interference caused by the P2*old* gene (5, 27). These four genes are widely distributed on the P2 genome and are representative of various classes of P2 genes. The results in Table 9 show that the eductants tested do not rescue the temperature-sensitive genes of the P2*ts* phage and do not interfere with λ. These data support the conclusion that the whole P2 genome has been deleted in the eductants.

Twenty independent eductants from strain AB311 were tested by R. Guzman and J. Dietrich for *mglP* activity. Table 10 shows that 14

TABLE 5. *Transduction by bacteriophage P1 to determine map order of gnd, his, and* $(P2)_H$

Donor: LG-106 = HfrH *thi* $(P2)_H$
Recipient: DF-412 = F⁻ *edd gnd str*ʳ *pyrD his*

gnd⁺ Selection			*his*⁺ Selection			*his*⁺ *gnd*⁺ Selection
Unselected markers	Observed frequency[a] (%)	Expected frequency[b] (%)	Unselected markers	Observed frequency[a] (%)	Expected frequency[b] (%)	
his⁺ (P2)	45	37.6	*gnd*⁺ (P2)	22.4	22.3	
his⁺ (—)	36	43.3	*gnd*⁺ (—)	49.4	49.5	
his⁻ (P2)	1.5	8.8	*gnd*⁻ (P2)	8.6	8.9	
his⁻ (—)	17.5	10.2	*gnd*⁻ (—)	19.3	18.4	
200 Transductants tested; CT[a] $(P2)_H$ = 46.5%[c]; CT *his*⁺ = 81%			196 Transductants tested; CT $(P2)_H$ = 31.1%[c]; CT *gnd*⁺ = 71.8%			200 Transductants tested; CT $(P2)_H$ = 52%[c]

[a] CT, cotransduction.

[b] Expected CT frequencies were calculated from the actual CT frequencies obtained for the two unselected markers taken separately.

[c] The cotransduction frequency of $(P2)_H$ with *his*⁺ selection is lower than that with *gnd*⁺ and *his*⁺ *gnd*⁺ selections and appears to be in variance with the deduced order *gnd*⁻ *his*⁻ $(P2)_H$. The lower value (31%) for CT of $(P2)_H$ with *his*⁺ selection seen here is probably not significant since CT frequencies for $(P2)_H$ with *his* range from 22 to 60% in other experiments.

TABLE 6. *Tests of eductants for parental markers*

Eductants	Growth without histidine	Reversion to histidine prototrophy	F'*his* crossing	Ability to grow on histidinol	Lysogenic for P2	Sensitive to P2 or P2*am*	Motility	Growth on α-l-glycerol phosphate (*glpT*)	6-Phosphogluconic dehydrogenase (*gnd*) activity	Shikimic acid permease (*shiA*)
I. All eductants in the series QE1-74 except those listed below	–	–	del[a]	–	–	+	+	+	–	
All eductants from K-12 strains	–	–	del	–	–	+	+	–[c]	–	
QE43, 44, 62	–	–	del	–	+[b]	–	+	+	–	
QE130-132	–	–	–[d]	–	–		+		–	
II. QE75-94	–	–			–					
QE95-129	–	–			–					+
III. LG-110	–	+		+	+	–	+	+	+	
LG-111	–	+		+	–	+	+	+	+	

[a] Indicates deletion of the six histidine genes tested.

[b] QE43 and 44 are eductants from a doubly lysogenic strain. QE62 is an eductant obtained from a plaque of P2 on K-40.

[c] The K-12 parent from which these eductants were isolated was also found to be *glpT*$^-$.

[d] No prototrophic growth was observed when an F'*his*$^+$ control strain was used. These strains are *recA* and therefore deficient in recombination.

TABLE 7. *Specific activities of enzymes*[a]

Strains	*rfb* Enzymes[b]			*gnd*
	rha-1	*rha-2*	*rha-3*	
LG-100	100	100	100	100
QE1	28	28	0	0
QE7	41	24	0	0
QE8	34	19	0	0
QE35	19	28	0	0
QE61	42	19	0	0
QE65	30	25	0	0
QE69	29	13	0	0

[a] Data from H. Nikaido (*personal communication*). Specific activities are expressed as percentage of the activity in the parent strain LG-110. Enzymes were assayed by the method described by Nikaido et al. (28). Residual activity (20 to 40%) of *rha-1* presumably represents the activity of uridine diphosphoglucose (UDPG) pyrophosphorylase toward UDPG. The situation is the same for *rha-2* (see reference 26). The low levels indicate the absence of *rha-1*, *rha-2*, and *rha-3* enzymes in the eductants tested.

[b] *rha-1*, Thymidine diphosphoglucose (TDPG) pyrophosphorylase; *rha-2*, TDPG oxidoreductase; *rha-3*, *TDP* rhamnose synthetase.

TABLE 8. *Ability of eductants to repair ultraviolet-irradiated* λ[a]

Plating strain	Genotype	Plaque-forming units/ml	
		Expt 1	Expt 2
K-40	*uvrC*$^+$	4.5×10^8	5.9×10^8
AB1157	*uvrC*$^+$	5.8×10^8	6.8×10^8
AB1884	*uvrC*$^-$	1.2×10^5	7.2×10^4
QE7		4.6×10^8	
QE8		7.1×10^8	
QE59		5.8×10^8	
QE61		5.0×10^8	
QE65		4.2×10^8	
QE23			5.7×10^8
QE35			7.3×10^8
QE37			4.1×10^8
QE39			4.2×10^8
QE41			5.0×10^8
QE57			4.4×10^8

[a] See Materials and Methods section.

eductants retained the parental level of methyl-β-D-galactoside uptake, whereas 2 eductants showed essentially no uptake and 4 eductants gave intermediate levels of uptake. The same results were obtained with 19 independent eductants obtained from a different strain: 11 showed 100% activity, 2 showed essentially no activity, and 6 showed intermediate levels of activity.

Table 6, part I shows that most of the eductants were able to grow with α-glycerol phosphate as the sole carbon source indicating that *glpT* was not deleted. A surprising finding was that strain K-12 was *glpT*$^-$. This strain was the wild-type *E. coli* K-12, transferred on nutrient

TABLE 9. *Prophage marker rescue and λ interference studies*

Strains tested	Plaques produced by			
	P2*ts40 vir24* gene B	P2*ts4 vir3* gene D	P2*ts13 vir3* gene H	λ*old*[a]
K-40	4[b]	0	0	+
LG-106	>1,500[c]	>1,000[c]	>1,500[c]	−
QE1	27[b]	0	0	+
QE7	8[b]	0	0	+
QE41	0	0	0	+
QE61	0	0	0	+
QE67	15[a]	0	0	−[d]

[a] + Indicates that λ is able to grow on the strain tested.

[b] The plaques appearing here are most likely due to revertants.

[c] Approximately 0.1% of the P2*ts vir* phage plated were able to form plaques on LG-106 at 42 C.

[d] QE67 is a λ lysogen and hence immune to superinfection by λ.

TABLE 10. *In vivo* D-[*methyl*-β-^{14}C] *galactoside uptake in eductants*[a]

Strain	Percentage of parental counts per min per 2 ml of cells[b]
AB311 (parent)	100
QE76-83, 86-88, 90, 92, 93	100
QE94	0.9
QE84	1.1
QE85	21.6
QE75	29.5
QE89	30.2
QE91	40.7

[a] These assays were generously performed by R. Guzman and J. Dietrich in the laboratory of M. B. Rotman.

[b] The measurements of accumulation of D-[*methyl*-β-^{14}C]galactoside in intact cells were performed with 2 ml of cells of about 10^9 cells/ml [325 μg (dry weight) per ml] by the method of Ganesan and Rotman (18). The activity of the parent strain was 8,700 counts per min per 2 ml of cells.

agar for more than 15 years in the culture collection of G. Bertani.

Strains resistant to high levels (40 μg/ml) of nalidixic acid were isolated from 10 eductants chosen at random. In addition, 20 independent eductants were isolated from a *nalA* mutant of LG-106. All retained the parental high resistance to nalidixic acid. These results indicated that the deletion does not include the *nalA* locus unless the loss of *nalA* is lethal to the cell.

Figure 1 shows the known extent of the deletion based on the results presented in Tables 6 to 10.

DISCUSSION

The combined data presented in Tables 6 to 10 indicate that eductants contain a deletion which covers the location H P2 prophage, *sbcB*, *his*, *gnd*, *rfb*, and in some cases *mglP*.

It is not known at present why some eductants give intermediate levels of methyl-β-D-galactoside uptake rather than 0 or 100% of wild-type activity. The possibility must be considered that *mglP* could be a complex locus containing genes governing several functions involved with methyl-β-D-galactoside uptake (M. B. Rotman, *personal communication*). If this is true, then it is possible that the three classes of uptake observed (Table 10) reflect at least three different end points of the deletions.

All of the eductants obtained from P2 lysogens were found to be nonlysogenic and sensitive to infection by P2 except for two which come from a doubly lysogenic strain and one from an infection experiment (Table 6). All eductants tested were unable to rescue *ts* phage markers (Table 9). No case of an eductant with a defective or normal prophage in location H was found. These data indicate that the whole prophage is deleted when eduction occurs.

The first known bacterial gene counterclockwise to $(P2)_H$ is *shiA*. This gene is present in those eductants tested (Table 6) as are all the other genes tested which are on this side of $(P2)_H$. Thus, it appears that one end of the deletion is closely determined and may be fixed at the counterclockwise hybrid attachment site (*see* Fig. 2). The location H attachment site appears to be altered due to the eduction event since it is not readily available for a P2 prophage upon lysogenization (34).

Comparison of the genetic maps of *E. coli* C and *E. coli* K (39) indicates that P2 location I in *E. coli* C corresponds approximately to the region of the *E. coli* K map where *mglP* is located (37; M. B. Rotman, *personal communication*). Additionally, histidine-linked P2 locations which are not allelic with location H or location I, but which are close to location I, have been shown to occur in hybrids produced by K to C and C to K transductions of the histidine region. In these hybrids, the normal location I or location H has been removed by the transduction (35). These histidine-linked prophage attachment sites are postulated to have "homology" for a region at or near the H site and possibly to be sites for action of the P2*int* gene product (34).

Our model for the eduction phenomenon (Fig. 2) postulates that eduction is an aberrant cross-

over event which requires the presence of a functioning P2*int* gene (34). Normally, during prophage excision (12), the two hybrid attachment sites (BP′ and PB′) interact and a single, reciprocal recombination event produces a freed, circular phage genome. When eduction occurs, however, the counterclockwise hybrid attachment site (PB′) would interact with one of the postulated "homology" regions near or within *mglP*, possibly corresponding to location I in *E. coli* C or to one of the alternate locations shown to exist in the hybrids. An *int*-promoted recombination event occurs and the portion of the bacterial chromosome from the P2 prophage to *mglP* is deleted.

The results presented here show that the host *recA* function is not required for eduction, whereas evidence is presented elsewhere (34) that eduction requires the presence of a functioning P2*int* gene. These lend support to the idea that recombination may be taking place between the hybrid site PB′ and secondary attachment sites for P2. It cannot be excluded, though, that there is only one site which covers the *mglP* gene. Here, one would have to assume that the reciprocal exchange can occur at several points within the site.

Eduction differs from the generation of λ particles capable of specialized transduction. With P2 the cell containing a deletion is recovered, whereas with λ a defective phage particle containing a piece of the bacterial chromosome is generated. In addition, λ-transducing particles are generated in the absence of *int* product (20), and the two crossover points are not restricted to a small number of sites (12), as is the situation with eduction. P2 particles capable of specialized transduction of *his* genes or *gnd* have not been found (Sunshine and Kelly, *unpublished results*; T. Blumenthal, *personal communication*). It seems probable that the piece of chromosome excised is too large to be accommodated within a P2 phage head.

Eduction appears to have some similarities to the reversion of the transducing phage λ*b2att*² to a nontransducing phage with the properties of the original phage parent λ*b2* [M. Shulman and M. Gotksman, *In* A. D. Hershey (ed.), The Bacteriophage, *in press*]. Phage λ*b2att*² arises by general recombination at homology regions outside the prophage attachment sites and includes at least two bacterial markers and the two hybrid attachment sites which bracket the prophage genome. The reversion of this transducing phage to the original nontransducing phage occurs when the phage *int-xis* system is functioning.

Two of the eductants described here are derived from a doubly lysogenic strain, indicating that eduction occurs in the presence of P2 repressor. This is in agreement with results presented elsewhere (10, 34) that *int* can function when P2 is repressed.

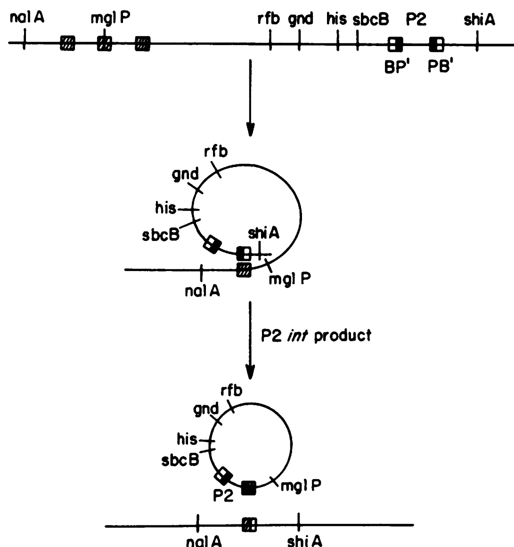

FIG. 2. *Model for the production of eductants. By the model proposed, the chromosome circularizes with the right hybrid phage-bacterium attachment site, PB′, pairing with one of the postulated homology regions near or within mglP. A single, reciprocal recombination event mediated by P2int product occurs cutting out a small segment of the chromosome. Markers are not drawn to scale.*

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