JOURNAL OF BACTERIOLOGY, Feb. 1974, p. 468-476

Vol. 117, No. 2
Printed in U.S.A.

Isolation of Specialized Transducing Bacteriophages for Gluconate 6-Phosphate Dehydrogenase (*gnd*) of *Escherichia coli*

RICHARD E. WOLF, JR., AND D. G. FRAENKEL

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115

Received for publication 22 October 1973

Specialized transducing phages for gluconate 6-phosphate dehydrogenase (*gnd*), a constitutive enzyme in *Escherichia coli*, have been isolated using a method previously described for other genes. The *gnd-his* region, carried on an F' episome, was first transposed to *tonB*. Rare phages carrying *gnd* were selected, by transduction, from ϕ80 lysogens of these strains; one phage also carried *his* (ϕ80*gndhis*). From the transductants, high-frequency transducing lysates were obtained; low multiplicity of infection then yielded defective lysogens. *tonB* deletion analysis of the ϕ80d*gndhis* lysogen shows the order of genes in the prophage to be *imm80...hisOGD...gnd;* according to a marker rescue experiment most phage late genes have been replaced by bacterial deoxyribonucleic acid. A heat-inducible, lysis-defective λ-ϕ80 hybrid derivative of ϕ80d*gndhis* has been prepared.

We have been interested in the mechanism of expression of genes for "constitutive" enzymes in *Escherichia coli*, particularly those of glycolysis and the hexose monophosphate shunt (D. G. Fraenkel and R. T. Vinopal, Annu. Rev. Microbiol, in press). For studying this problem, certain types of experiments require deoxyribonucleic acid (DNA) enriched for the gene in question. Such a reagent (usually obtained from specialized transducing phage) would facilitate specific assay of messenger ribonucleic acid and of its in vivo half-life, as well as in vitro experiments on transcription and translation.

In this paper we report the isolation of specialized transducing phages carrying the gene, *gnd*, for gluconate 6-phosphate dehydrogenase (6-phospho-D-gluconate: nicotinamide adenine dinucleotide phosphate oxidoreductase [decarboxylating], EC 1.1.1.44), a constitutive enzyme of the hexose monophosphate shunt (see Fig. 1); one of the phages also carries the histidine operon (*his*). A preliminary report of this work appeared previously (R. E. Wolf, Jr., and D. G. Fraenkel, Fed. Proc. 1972, p. 444). Independent isolations of histidine transducing phages carrying similar regions of the *Salmonella typhimurium* (31) and *E. coli* (2) chromosome have been reported.

MATERIALS AND METHODS

Media and growth conditions. Minimal medium 63 (25) was supplemented with the carbon source (4 mg/ml), thiamine-hydrochloride (1 μg/ml), and, as specified, amino acids and uracil (each 25 μg/ml), streptomycin sulfate (100 μg/ml), rifampin (Schwarz-Mann Co., 100 μg/ml), citrate (1.25 mg/ml), and agar (2%). F top agar (7.5 g of agar per liter, 8 g of NaCl per liter [16]) was used in transductions. Gluconate tetrazolium indicator plates were described earlier (8). The standard broth was medium 63 supplemented with 1% tryptone (Difco) and 0.4% yeast extract (Difco). The standard rich plate was tryptone-yeast extract (TYE) agar (9). LB broth (16) was used for growing ϕ80 lysogens prior to phage induction. LBC broth (LB broth supplemented with 2.5 mM $CaCl_2$ [13]) and plates (1.5% agar) were used for preparing and titering lysates of P1kc. H plates and H top agar (9) were used for ϕ80 phage titrations. Lysates of colicins V and B were made from cells grown in B broth (23). Growth conditions were aerobic and at 37 C unless otherwise specified.

Scoring and selection of gnd. Gluconate 6-phosphate is metabolized in two ways (Fig. 1): via *gnd* and the hexose monophosphate shunt, and also via gluconate 6-phosphate dehydrase (*edd*) and the inducible Entner-Doudoroff pathway (D. G. Fraenkel and R. T. Vinopal, Annu. Rev. Microbiol., in press). Thus, selection and scoring of *gnd* is done most easily in *edd*$^-$ strains. With gluconate as carbon source, *gnd*$^-$, *edd*$^+$ strains grow at almost wild-type rate, *gnd*$^+$, *edd*$^-$ strains grow slowly, and *gnd*$^-$,*edd*$^-$ strains do not grow. Gluconate tetrazolium indicator plates can be used to distinguish *gnd*$^+$ *edd*$^-$, *gnd*$^-$ *edd*$^-$, and *edd*$^+$ strains (18).

Bacterial strains. Bacterial strains are listed in Table 1, and the positions of relevant markers on the *E. coli* genetic map are shown in Fig. 2.

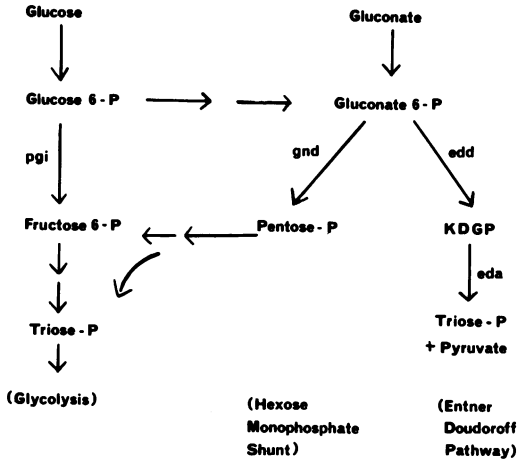

FIG. 1. *Pathways of metabolism. Abbreviations: pgi, phosphoglucose isomerase; gnd, gluconate 6-P dehydrogenase; edd, gluconate 6-P dehydrase; eda, 2-keto-3-deoxygluconate 6-P (KDGP) aldolase.*

RW10, an *edd*$^-$, *gnd*$^-$, φ80-sensitive strain was prepared as follows. An eductant (deletion of the *gnd-his* region [12]) was selected from strain DF1647; the resulting gluconate-negative (*edd*$^-$,*gnd*$^-$) strain was, like its parent, resistant to φ80. It was known that the strain carried a *tonA* mutation, but there was also some uncertainty about the *tonB* region. Therefore, both regions were replaced. First, a conjugation was done with a (*tonB-trp*)∇ derivative of strain CA8000, with selection of *pyrD*$^+$,*str*^{r} and scoring for *colVcolB*^{r}, φ80*h*^{s}, *trp*$^-$ (i.e., *tonA*$^+$, *tonB*$^-$). Then the *tonB* region was repaired by P1 transduction from strain CA8000 by selection of *trp*$^+$. All recombinants (e.g., strain RW10) were sensitive to φ80*v* and to the colicins (i.e., *tonB*$^+$).

The F'$_{ts}$*his*$^+_{402}$ episome of strain TM131 was transferred to strain RW10 by conjugation, for 1 hr at 34 C with selection of *his*$^+$ F-ductants (e.g., strain RW11) on lactose minimal medium containing tyrosine at 30 C (lactose counterselected the donor strain, which is *S. typhimurium*).

RW75 and its derivatives (e.g., strain RW78 and RW84) were nonreverting, gluconate-negative strains (*gnd* deleted and two mutations in the Entner-Doudoroff pathway [*gnd*$^-$, *edd*$^-$, *eda*$^-$]) used for selection of the rare *gnd*$^+$ transducing phages. First, a revertant on gluconate (*edd*$^-$, *eda*$^-$, see reference 4) was selected from strain DF1671 (*eda*$^-$); then a *gnd* deletion was introduced by P2 eduction, giving strain RW75. Strain RW78 is a φ80h lysogen of strain RW75, and strain RW84 is a *pgi*$^+$ revertant of strain RW75 (selected on glucose minimal medium containing histidine).

Bacterial matings. Screening of transposition strains to determine the origin and direction of transfer was done using as recipient strain X'121*rif*^{r}, with selection on appropriately supplemented glucose plates containing rifampin (to counterselect the donors). Aluminum plates (9.5 cm in diameter) with an array of twenty 1-cm wells were used. Each well was inoculated with 0.1 ml of broth, 0.02 ml of donor culture (grown two doublings from an inoculum of 5 × 10^7/ml), and 0.10 ml of X'121*rif*^{r} (2 × 10^8/ml in exponential phase). After 75 min of incubation at 37 C the mixtures were replicated to selective medium. *pyrD*$^+$ and *trp*$^+$ recombinants were scored after incubating the selective medium at 37 C for 36 h; *his*$^+$ recombinants were determined after incubation of the selective medium at 42 C, a condition that counterselects growth of *his*$^+$ F-ductants.

Chromosomal transfer by the transposition strains was quantitatively estimated by conventional flask matings (11). *pyrD*$^+$, *trp*$^+$, and *his*$^+$ recombinants were scored under the selective conditions used in the initial screening. *gnd*$^+$ transfer was determined in flask matings with strain DF710*rif*^{r}, incubating the appropriately supplemented gluconate minimal medium at 42 C (to counterselect growth of *gnd*$^+$ F-ductants).

Bacteriophages. Table 2 lists the bacteriophages used.

Preparation of lysates. φ80*v* was prepared by the plate method (1). Phages (10^5) were incubated with 10^7 exponential-phase strain CA8000 at 37 C for 20 min, and the mixture was plated on fresh H plates in 2.5 ml of H top agar and 1 ml of B broth. After overnight incubation at 37 C the top agar was resuspended with vigorous mixing in a small volume of B broth, chloroformed, and centrifuged at low speed. Titers in the supernatant fluids were 2 × 10^{11} to 5 × 10^{11}/ml.

Ultraviolet light induction of lysogens was used to prepare stocks of φ80, φ80*h*, λ*h80*, φ80*dgndhis*, and λ*h80dgndhis*. Lysates containing λ*cI857St68h80* were made by growing lysogens (strain JG33 or RW134) in B broth or LB broth to 2 × 10^8/ml, heating in a water bath at 42 C for 15 min, with very vigorous aeration, and incubating for 4 to 5 h at 37 C before treating with chloroform; such lysates are conveniently titered on strain M182, a λ*i*^{434} lysogen whose prophage provides the *S*$^+$ function necessary for plaque formation.

Low-frequency transducing lysates were prepared from φ80*h* lysogens of the transposition strains as follows (adapted from E. B. Konrad, Ph.D. thesis, Harvard Univ., Cambridge, Mass., 1969). Clones were inoculated to LB broth containing gluconate (10 mg/ml) and grown for two to three divisions at 42 C to 2 × 10^8/ml. The cells were collected, resuspended (4 × 10^8/ml) in 0.1 M $MgSO_4$, and irradiated as a 0.5-cm layer for 60 s with a 15-W General Electric germicidal lamp (G15T8) at a distance of 70 cm. Irradiated cultures were diluted with an equal volume of fresh broth and grown 3 to 4 h with very vigorous aeration in dark flasks (we covered ordinary flasks with foil). After 15 min of chloroform treatment (5 ml/100 ml), the debris was removed by centrifugation-yielding lysates with titer of 1 × 10^{10} to 2 × 10^{10}/ml. The lysates were concentrated using a phase system (S. Gottesman, Ph.D. thesis, Harvard Univ., Cambridge, Mass., 1970; adapted from reference 19). NaCl, dextran-SO_4 500 (Pharmacia), and polyethylene glycol 6000 (Schwarz-Mann) were added to 1.75%, 0.21%, and 6.9% (wt/vol), respectively, and the mixture (e.g., 200 ml) was slowly stirred at 4 C to dissolve. After

TABLE 1. *Bacterial strains (E. coli K-12)*[a]

Strains (in order of use)	Genotype	Source or reference
TM131	F'$_{ts}$*his*$^{+}_{402}$/*hisB22 str*^{r} (*S. typhimurium*)	F. Blasi (2)
DF1647	F^{-} *edd-1 galK35 his-68 mtl-2 pyrD34 str-125 thi-1 tonA22 tyrA2 xyl-7*	This laboratory (18)
CA8000	HfrH *rel-1, thi-1*	J. Beckwith
RW10	F^{-} *edd-1* (*galK35?*) (*his-gnd*)$\overset{P2}{\nabla}$ *mtl-2 str-125 thi-1 tyrA2 xyl-7*	See Materials and Methods
RW11	F'$_{ts}$*his*$^{+}$*gnd*$^{+}_{402}$/*RW10*	See Materials and Methods
X'121*rif*^{r}	F^{-} *galK35 his-68 mtl-2 pyrD34 rif*^{r} *str-125 thi-1 trp-45 tyrA2*	*rif*^{r} mutant of strain X'121 (J. Beckwith)
DF710*rif*^{r}	F^{-} *edd-1 gnd-1 mtl-2 pyrD34 rif*^{r} *str-125 thi-1 tonA22 tyrA2 xyl-7*	*rif*^{r} mutant of DF710 (18)
Type I and type II transpositions		See results
DF1671	F^{-} *eda-1 his*$^{-}$ *pgi-2 str*^{r}	This laboratory (4)
RW75	F^{-} *eda-1 edd*$^{-}$ (*his-gnd*)$\overset{P2}{\nabla}$ *pgi-2 str*^{r}	See Materials and Methods
RW78	RW75(*ϕ80h*)	See Materials and Methods
RW84	F^{-} *eda-1 edd*$^{-}$ (*his-gnd*)$\overset{P2}{\nabla}$ *str*^{r}	See Materials and Methods
RW100	RW78 (*ϕ80dgndhis, ϕ80h*)	See Results
RW101-RW114	RW78 (*ϕ80dgnd*$_{1}$-*ϕ80dgnd*$_{14}$, *ϕ80h*)	See Results
RW115	RW75 (*ϕ80dgndhis*)	See Results
RW130, RW159	RW75 (λh80, λ*h80dgndhis*)	See Results
RW134	RW75 (λ*cI857St68h80*, λ*cI857St68h80dgndhis*	See Results
Other strains		
JG33	HfrH *araD*$^{-}$ *leu*$^{-}$ *thi*$^{-}$ (λ*cI857St68h80*)	E. B. Konrad
M182	F^{-} *galK*$^{-}$ *galU*$^{-}$ *lac*$^{-}$ *str*^{r} (λi^{434})	E. B. Konrad
W3110	F^{-} *str*^{s}	L. Soll
X178	F' *trp*$^{+}$ *colV colB/his*$^{-}$ *lac*$^{-}$ *thi*$^{-}$ *str*^{r}	J. Beckwith

[a] Almost all genetic symbols are in Taylor and Trotter (30). The notation (*his-gnd*)∇ is used to indicate deletions induced by phage P2 (12). When possible, allele designations are those supplied by the *E. coli* Genetic Stock Center at Yale University. All known bacterial mutations carried by a strain have been included in this table, with some cases of uncertainty noted.

standing overnight in a cylinder, at which time the upper phase appeared clear and the lower phase appeared turbid, most of the upper phase was removed by aspiration and the remaining material was resuspended and centrifuged at 4,000 × *g* for 15 min. The pellet was resuspended in 2.5 ml of 0.1 M sodium phosphate, 0.01 M $MgSo_4$, pH 7.0, and dextran-SO_4 was precipitated by slow addition of KCl to 1 M and removed by centrifugation. The supernatant fluid was dialyzed versus two changes of 100 volumes of the phosphate buffer. Addition of chloroform (0.05 ml/ml) to this fraction gave further precipitation, yielding a final supernatant fraction with a 20-fold increase in titer over the starting material and a 70% yield. (A simpler method for lysate concentration has recently been described [32].)

Lysates of P1kc were prepared by adding phage at a multiplicity of infection (MOI) of 0.01 to exponential-phase cells in LBC broth at 5 × 10^7/ml and continuing incubation until lysis. Lysates were treated with chloroform and titered on strain W3110.

Colicin V and B were prepared from strain X178 (9).

Specialized transduction. Recipient cells were grown in LB broth to 5 × 10^8/ml, harvested, resuspended in 0.05 volumes of 0.01 M $MgSO_4$, and incubated for 1 h at 37 C. For transduction at high MOI (1 to 2 for nonlysogenic recipients and 10 to 20 for homoimmune lysogens) with lysates of *ϕ80h* or *λh80*, 0.1 ml of phage and 0.1 ml of cells were mixed, incubated for 20 min at 37 C, and plated with 2.5 ml of F top agar on the selective medium. Transductions at low MOI were similar, but the high-frequency transducing (HFT) lysates were first diluted in 0.01 M $MgSO_4$. When the transducing phage carried the λ*cI857* allele, incubations were at 34 C.

HFT lysates of the *ϕ80dgnd* phages were identified by rapid screening tests. Wells contained 0.02 ml of lysate and 0.10 ml of recipient cells, and after 30 min at 37 C the mixtures were replicated to selective plates. HFT lysates gave confluent areas of growth at 37 C.

Other phage techniques. Defective lysogens of the *ϕ80dgnd* phages were identified by a halo test (E. B. Konrad, personal communication). Clones were transferred to small areas of an H plate (50 per plate)

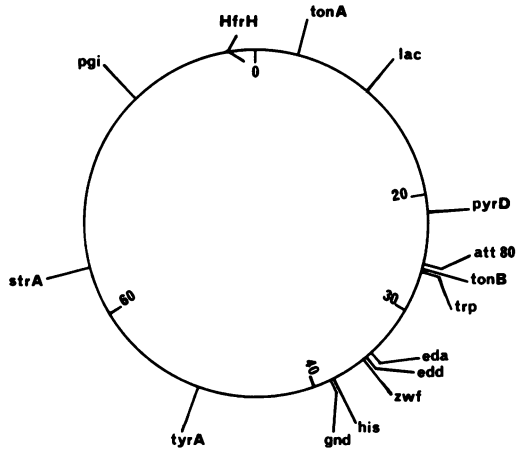

FIG. 2. *E. coli genetic map (30).*

TABLE 2. *Bacteriophages*

Name	Comments and source
P1kc	L. Gorini
P2	S. E. Luria
P2c_5nip_1	Temperature-inducible derivative of P2 (27); M. Sunshine
φ80	J. R. Beckwith
φ80*h*	Host-range mutant of φ80 able to infect *tonB* mutants; E. B. Konrad
φ80*v*	J. R. Beckwith
φ80*am*-x	Amber mutants of φ80 in genes 1 to 19 (20); L. Soll
λ*h80*	φ80 with early genes of λ (reference 22, called hy5 in reference 29); S. Gottesman
λ*cI857St68h80*	Derivative of λ80 which is heat-inducible and lysis defective; from strain JG33

and incubated for 3 h at 37 C. After exposure to ultraviolet light, the irradiated clones were transferred to a second H plate spread with 10^8 cells of strain CA8000. Active phages released from double lysogens formed halos (areas of lysis) and defective lysogens did not.

Curing of the φ80*dgnd* defective lysogens (adapted from reference 24) was achieved by streaking to a gluconate tetrazolium plate previously spread with 10^9 λ*h80;* a control plate contained no phage. Curing was scored as increased number of *gnd*$^-$,*edd*$^-$ clones on the former plate, as recognized by size and color.

Phage genes missing in φ80*dgndhis* were determined in the defective lysogen strain RW115 using a set of amber mutants in a marker rescue experiment by the method of Schlief et al. (21).

The cross between λ*h80* and φ80*dgndhis* was done by making a plate lysate of λ*h80* on strain RW115 (as in making lysates of φ80*v*). λ*cI857St68h80* was crossed with λ*h80dgndhis* by adding it at an MOI of 3 to an ultraviolet light-induced culture of strain RW159, and a plate lysate was prepared.

Transduction with P1kc was as described earlier (5) with an MOI of 0.75.

Eductants were made with P2 or P2c_5nip_1, as described previously (6).

Selection of tonB mutants. The technique of Gottesman and Beckwith (9) was used. Clones were grown to saturation in LB broth, and the cells from 2.5 ml were collected by centrifugation and resuspended in the remaining drops of broth. Four-tenths milliliter of φ80*v* lysate and 0.4 ml of colicin lysate were added (both *tonB* and *tonA* mutants are resistant to φ80*v*, but *tonA* mutants are sensitive to the colicins), and after 20 min at 37 C the mixture was spread on two gluconate tetrazolium indicator plates and incubated.

RESULTS AND DISCUSSION

Specialized transducing phages usually derive from strains in which the gene of interest is near an inducible lambdoid prophage. We arranged this situation for *gnd* using a method described by *lac* (3) and *ara* (9). A strain was prepared that had a deletion of *gnd* on the chromosome, and carried *gnd*$^+$ on an F′ episome whose replication was temperature sensitive. The episome was then integrated into *tonB* by selecting simultaneously for *tonB* mutation and *gnd*$^+$ expression at the nonpermissive temperature. Then, the transposition strains were lysogenized with φ80*h*, induced, and (rare) φ80*dgnd* phages were selected by transduction.

The original reason for choosing *gnd* was the relative ease of preparing useful strains. *Gnd* deletions were known in enterobacteriaceae (17, 18) and could also be selected by P2 eduction (e.g., 6, 26). Selection of *gnd*$^+$ requires a recipient to be *gnd*$^-$,*edd*$^-$ (i.e., a strain with no pathway for metabolism of gluconate 6-phosphate, Fig. 1). At the time, it was not known that deletion of *edd* was also possible (7), but we were able to use a nonreverting strain for detection of the very rare φ80*dgnd* phages. In addition, *E. coli* F′ episomes carrying *gnd* were known (see reference 15 for a recent list). Indeed, it seemed likely that a temperature-sensitive, *gnd*-carrying episome also existed, since *gnd* is approximately 50% linked in P1 transduction with *his* (18), and a temperature-sensitive episome carrying *his*$^+$ had been constructed (F. Blasi and J. Miller, personal communications; reference 2). This episome, F′$_{ts}$*his*$^+_{402}$, was derived, in part, from F′*his*$^+$80 (2; also called F57 [15]), an episome known to carry *gnd* (17). Although the origin of the F′ *his* involved transfer in *S. typhimurium* (J. Roth, personal communication), it probably was derived from *E. coli* strain AB311 (15), and has been shown to code for the *E. coli* gluconate 6-phosphate

dehydrogenase (14). We could show (see Materials and Methods) that the episome of strain TM131 (generously supplied by F. Blasi) indeed carried *gnd*$^+$, and thus constructed strain RW11: F'$_{ts}$ *his*$^+$*gnd*$^+_{402}$/*edd*$^-$ (*his-gnd*) ∇, *tyrA*$^-$ which could be used for selection of strains with *gnd* transposed to near *tonB*.

Transposition of gnd to tonB. Strain RW11 depends on episomal genes for gluconate utilization and for histidine biosynthesis. At 42 C the nonpermissive temperature for replication of the episome) such a strain should be unable to grow on gluconate unless the episome has become integrated in the chromosome. The fraction of (30 C grown) cells which formed colonies at 42 C on gluconate minimal plates (with tyrosine, and with or without histidine) was 2 to 3%. On gluconate tetrazolium indicator plates, as many colonies were formed at 42 C as at 30 C, but 97 to 98% of them were red and small, the appearance characteristic of *gnd*$^-$, *edd*$^-$ strains (18) (i.e., the heat-cured segregants); the other colonies appeared to be *gnd*$^+$, *edd*$^-$ (red, but not small) and grew on gluconate or glucose minimal plates with or without histidine (i.e., Hfr's). (We noticed also that only 2 to 3% of RW11 cells formed colonies at 42 C on glucose minimal medium [with tyrosine and histidine]; on this medium there was no obvious selection for expression of episomal genes, and the failure of most cells to grow may be a transdominant effect of temperature-sensitive episomal replication on minimal medium.)

Thus, the background of spontaneous integration of the episome in strain RW11 was rather high. (As noted below, this integration may be near the chromosomal *his-gnd* or *lac* regions.) Therefore, if spontaneous *tonB* mutants were selected in strain RW11 it was expected that 2 to 3% would be *gnd*$^+$ at 42 C because of integration by an independent event. Nonetheless, we hoped to find a *gnd*$^+$,*tonB* mutant which had arisen by a single event, the inactivation of *tonB* by integration of the episome into it. (The integration of F'$_{ts}$*ara* at *tonB* was found in spite of the high background of integration at the chromosomal *ara* locus [9].)

Therefore, 35 independent cultures of strain RW11 were treated for selection of spontaneous *tonB* mutants (see Materials and Methods) and spread on gluconate tetrazolium indicator plates at 42 C. The frequency of survivors was about 10^{-7}, and 2 to 3% were apparently *gnd*$^+$, *edd*$^-$ integrands (red, but not inhibited, colonies); both frequencies varied widely between different cultures. Two to five *gnd*$^+$,*tonB*$^-$ clones from each selection were purified on gluconate tetrazolium plates at 42 C, care being taken to choose colonies of varying appearance. Those which appeared relatively stable (e.g., less than 25% segregation of *gnd*$^-$,*edd*$^-$ clones) were then mated with strain X'121*rif*^{r} (his$^-$, *trp*$^-$,*pyrD*$^-$) to determine whether any were Hfr's with origin of transfer near *tonB*, and, if so, the direction of chromosomal transfer (see Materials and Methods, and Fig. 3).

Twenty-five of the 37 isolates tested evidently did *not* have F' integrated near *tonB:* they transferred *his* at high frequency and *pyrD* and *trp* at lesser and approximately equal frequency. These Hfr's probably arose by episome integration independent of *tonB* mutation. The site(s) of integration are not known, but might be in the *his-gnd* or *lac* regions. (For example, integration near *his* would be expected if the episome contained more bacterial DNA from this region than was deleted in the eductant. Or, considering that the F'$_{ts}$*his-gnd* episome was obtained in a cross between F'$_{ts}$*lac* and F'*his-gnd*, it might contain sequences near *lac* even though it does not carry *lac*$^+$.) We note that when the *tonB* selection was plated on gluconate minimal plates containing tyrosine and citrate (instead of gluconate-tetrazolium plates), all isolates (110/110) from 40 selections were of this undesired class.

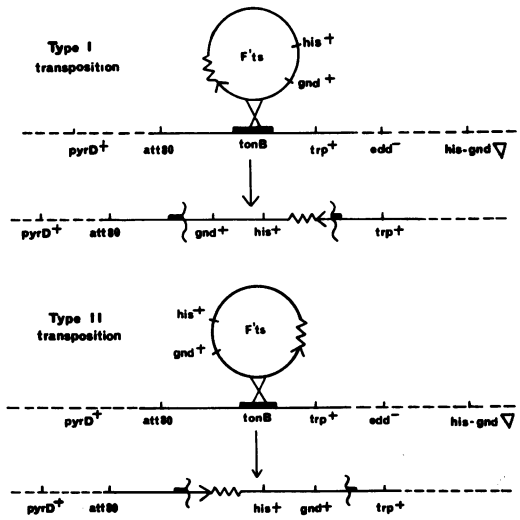

FIG. 3. *Transpositions. If integration of the episome occurred at tonB by a single crossover, there are six possible structures with respect to origin of transfer and position of gnd and his. The two classes identified by the conjugation experiments are probably type I and type II. (The episome structure is written as origin-gnd-his because this is the sequence in the probable progenitor strain AB311. Symbol:* ∧∧←, *depicts the origin and orientation of transfer.*

Twelve of the tested 37 isolates from the gluconate tetrazolium plate selections were Hfr's with origin of transfer near *tonB*. They were found in five of the selections as the sole class of isolate (two to four isolates tested from each selection). In two of the selections, the Hfr's transferred *trp*, but not *pyrD* or *his* in a 1-h mating period (i.e., type I transpositions, Fig. 3), whereas in three others, *pyrD* but not *his* or *trp* were transferred (type II transpositions, Fig. 3). The origin and direction of transfer was confirmed for these isolates in conventional flask mating experiments. The two transposition types of interest were also tested by mating with a different F⁻ (DF710*rif*^{r}), and neither type transferred *gnd* in a 1-h mating period. These results show that integration of the episome near *tonB* did occur in strain RW11, at a frequency of about 10^{-10}, with apparently two different transposition types recovered. If the episomal integration occurred by single crossover, then the relative orientations of *his* and *gnd* with respect to the chromosome are normal in type II strains and inverted in type I strains.

The transposition strains of interest were rather unstable. For example, in one experiment, growth in broth at 42 C was followed for four successive doublings by plating on gluconate tetrazolium plates; the frequency of *gnd*⁻, *edd*⁻ segregants was approximately 10^{-1} at each doubling. The fraction of segregants could be much reduced if the strain were grown in broth containing gluconate, presumably because its growth would be inhibited.

Since there were no obvious differences between independent isolates of type I and type II transpositions strains, one of each type was used for the phage selections.

Isolation of gnd⁺ specialized transducing phages. The type I and type II transposition strains were infected with phage φ80*h* (the host range allele, *h*, allows infection of *tonB* mutants) and lysogens isolated (Fig. 4, first line). Chromosomal transfer in the lysogens was confirmed (as above) using as recipient a φ80*h* lysogen of strain X'121*rif*^{r}. The lysogens were induced with ultraviolet light, and the lysates were concentrated (see Materials and Methods). The concentrated lysates were used for infection (MOI of 15 to 20) of a nonreverting gluconate-negative strain, RW78 ((*his-gnd*)∇, *edd*⁻, *eda*⁻, φ80*h*). Transductants were selected at 37 C on gluconate minimal medium containing histidine. In all cases, the transductants appeared after 4 to 5 days of incubation. From two lysates of type I strains, 8 *gnd*⁺ transductants were found, at a frequency of 10^{-11} transductants per plaque-forming unit (PFU); one of the eight also proved to be *his*⁺. Four other lysates of the type I strain yielded no *gnd*⁺ transductants (from approximately 10^{12} phage). From three lysates of the type II strain, a total of seven *gnd*⁺ transductants were found, also at a frequency of 10^{-11}, and two other lysates failed to yield transductants (from approximately 10^{12} phage).

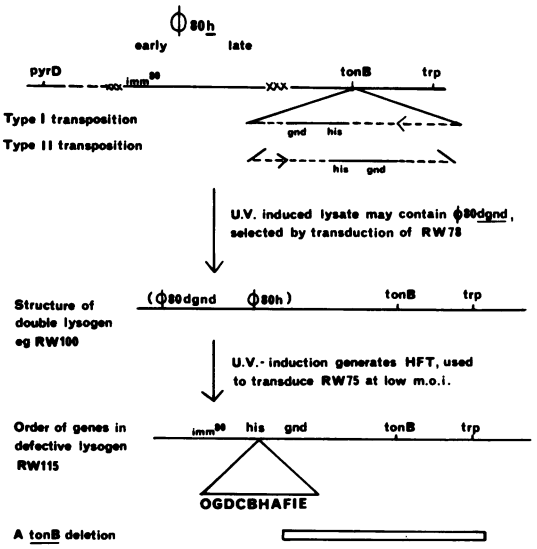

FIG. 4. *Selection and structure of φ80dgnd phages. Notation: ×××, hybrid attachment regions; - - -, possible locations of F factor genes and other episomal DNA; parentheses around φ80dgnd, φ80h indicate unspecified order of the defective and wild-type prophages. The particular tonB deletion indicated is that of RW118: gnd⁻, his⁻, hol⁺, trp⁻, and the actual ends are not mapped.*

After purification of the transductants (RW100-RW114) on the selective medium (2 to 3 days of incubation, as with normal *gnd*⁺, *edd*⁻ strains) they were induced and the lysates were used in a spot test for *gnd*⁺ transduction of strain RW78. All 15 lysates were HFT, giving confluent growth of *gnd*⁺ transductants; the lysate from the original *gnd*⁺, *his*⁺ transductant strain (RW100) was also an HFT for *his*⁺. In these HFTs the titers of PFU were 2×10^9 to 4×10^9/ml, and transducing titers were 10^{-3}–10^{-4} per PFU using low multiplicities of infection and the lysogenic recipient RW78.

To make strains lysogenic for only the specialized phages, each HFT was used to transduce nonlysogenic recipients (RW75 or RW84) at low MOI to growth at 42 C on gluconate minimal plates with histidine. (Reinfection of

the transductant clones by normal phage released by other cells is prevented at this temperature [9].) The frequency of transduction was 10- to 20-fold less than with the lysogenic recipients. Transductants from the lowest MOI (10^{-3} to 10^{-4}) were purified on gluconate tetrazolium indicator plates at 42 C. Most transductants were very unstable, segregating many gluconate-negative clones. Relatively stable clones, however, were found among transductants from nine different HFTs (four of ultimate type I-origin and five from type II). Some of these were defective lysogens, presumably integrated at *att*ϕ*80*, according to the following tests: (i) ultraviolet light induction (halo test, see Materials and Methods) did not yield active phage, but induction in the presence of helper gave HFT's for *gnd;* (ii) superinfection with λ*h80* (25; see Materials and Methods) increased the frequency of *gnd*$^-$, *edd*$^-$ segregants; and (iii) *tonB* deletion analysis (see below).

Genetic structure of ϕ80dgndhis. The ϕ80*dgndhis* phage was chosen for further study because the presence of a second bacterial marker (*his*) on it could be useful later. First, we showed by enzyme assay that the defective lysogen carrying this phage (RW115) actually did contain gluconate 6-phosphate dehydrogenase activity. Then, to determine which phage genes had been replaced by bacterial DNA in ϕ80*dgndhis*, a marker rescue experiment was done. Strain RW115 was treated with ultraviolet light to destroy phage immunity and infected with a series of ϕ80 phage carrying amber mutations in various genes (kindly supplied by L. Soll). Active phage is made only if the prophage can supply the function missing in the ϕ80 amber mutant. It was found that ϕ80*dgndhis* carries ϕ80 genes 15, 14, 16, 17, 18, 19, 1, 2, and 3 (likely corresponding to lambda genes N, O, P, Q, R, A, W, and B, respectively [20]); it did not complement ϕ80 genes 4 to 13 (other head and tail genes). It must carry *imm80*, since ϕ80*c* does not plaque on RW115 (and ϕ80*vir* does). Thus, in ϕ80*dgndhis* bacterial DNA replaces most of the ϕ80 late functions.

The overall order of *gnd*, *his*, *imm80*, *tonB*, and *trp* was shown by *tonB* deletion analysis of RW115. *tonB* mutants were selected as usual and plated on gluconate tetrazolium plates at 37 C. 135 *gnd*$^+$, *tonB*$^-$ and 135 *gnd*$^-$, *tonB*$^-$ mutants were purified on the same medium and tested for dependence on histidine and histidinol. (Appropriately supplemented glucose minimal plates containing citrate were used for this, since *tonB* mutants require citrate for uptake of iron on minimal plates [9].) All of the *gnd*$^+$, *tonB*$^-$ set were *his*$^+$. Only 1/135 *gnd*$^-$, *tonB*$^-$ clone was *his*$^+$, but 6/134 *his*$^-$ clones could grow with histidinol; some of the latter isolates were *trp*$^-$. All *gnd*$^-$*tonB*$^-$ isolates that could grow with histidinol retained *imm80*. These results show the overall order to be *imm80-his-gnd-tonB-trp* (Fig. 4) with the *his* operon probably oriented as shown (mutants lacking distal *his* operon genes can use histidinol, its dehydrogenase being the product of *hisD*). The direction of *gnd* transcription is not known.

Formation of ϕ80*dgndhis* was a very rare event, which may have involved both an aberrant phage excision and a shortening of the distance between these genes and ϕ80*h* (perhaps by a chromosomal inversion, since it came from a type I transposition strain). Gottesman and Beckwith (9) suggested that such events might have occurred in the formation of ϕ80*dara*. Considering the rarity of the events it is possible that some of the other *gnd* transducing phages have different genetic structures (e.g., replacement of other phage genes, or inversion).

Construction of λcI857St68h80dgndhis. To facilitate making lysates, we introduced into the ϕ80*dgndhis* the lambda mutation cI857 (28), which specifies temperature-sensitive repressor. We also put in the lambda mutation St68 (10) which (i) prevents lysis of the host and thus allows increased amounts of phage gene products to accumulate in induced cells, and (ii) eases the construction of defective lysogens by preventing phage release and secondary infection. With ϕ80*dara* (S. Gottesman, Ph.D. thesis, Harvard Univ., Cambridge, Mass., 1970), and ϕ80*dpfkA* (A. T. E. Morrissey, Ph.D. thesis, Harvard Univ., Cambridge, Mass., 1971) heat-inducible, lysis-defective derivatives had been difficult to obtain in a single vegetative cross with λ*cI857St68h80*. We therefore used a two-step procedure (S. Gottesman, Ph.D. thesis, Harvard Univ., Cambridge, Mass., 1970) first forming the hybrid-defective phage λ*h80dgndhis* and then introducing the other lambda mutations (Fig. 5). The defective lysogen RW115 (ϕ80*dgndhis*) was infected with λ*h80*, and the lysate was used at low MOI (10^{-2} to 10^{-4}) to transduce strain RW75 to growth on gluconate-minimal medium at 42 C. The efficiency of transduction was 10^{-6}. Three of 44 transductants (e.g., RW130) from the highest dilution plates proved to be double lysogens carrying both λ*h80* and λ*h80gndhis*: (i) they were sensitive to ϕ80*c* (and thus lacked ϕ80 immunity); (ii) when induced with ultraviolet

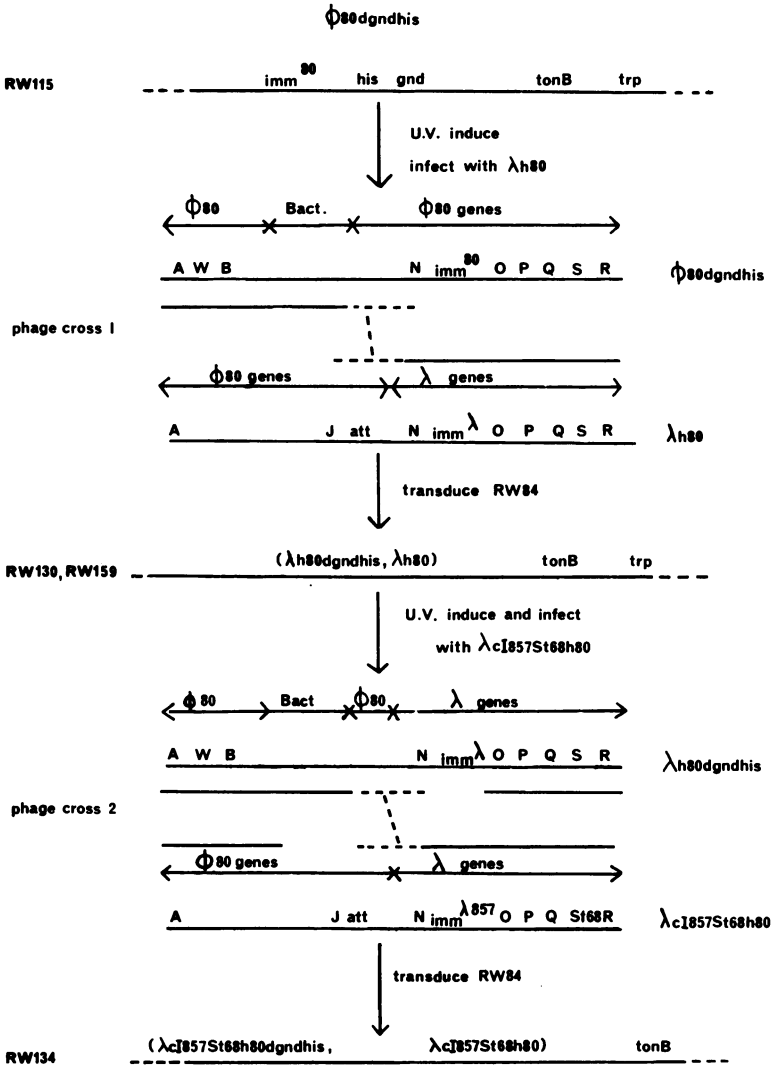

FIG. 5. *Construction of λcI857St68h80dgndhis. To show homology, φ80 genes are named like analogous λ genes.*

light all gave phages which formed plaques on φ80 lysogens and nonlysogens at the same frequency, but did not plaque on a *λh80* lysogen; (iii) the lysates all transduced strain RW84 to growth on gluconate at 42 C (e.g., strain RW159) at low MOI at efficiencies of 5×10^{-5}. That is, they were HFT's for *gnd* and *his*. No transductant from this cross was singly lysogenic for *λh80dgndhis*, since all gave active phage upon induction.

One of the double lysogens (strain RW159) was induced with ultraviolet light and infected with *λcI857St68h80* (MOI of 3); the lysate was used to transduce RW84 (MOI of 10^{-2} to 10^{-4}) to growth on gluconate at 30 C. Of 148 transductants purified from the selective medium, 47 were unable to grow on this medium at 42 C, and thus contained *λcI857* and not λcI^+. All 33 of 33 that were heat induced in LB broth failed to lyse in 4 h [whereas a control, RW84, lysogenic for *λcI857h80*(S^+) lysed in 90 min], but when then treated with chloroform, they gave lysates which were HFT for both *gnd* and *his*. These HFT's contained a phage which formed plaques on M182 (a λi^{434} lysogen whose S^+ function is transactivated by the superinfecting phage) but not on the nonlysogenic strain CA8000. The 33 transductants (e.g.,

strain RW134) were therefore lysogens carrying both λ*cI857St68h80* and λ*cI857St68h80dgndhis*.

ACKNOWLEDGMENTS

We are grateful to Anna Woodlock for expert technical assistance.

This work was supported by grants from the National Science Foundation (GB15958 and GB32284x) and the American Cancer Society (BC-90). R.E.W., Jr., is a postdoctoral fellow (GM46972) and D.G.F. a career development awardee (5-K3-GM7344) of the Public Health Service National Institutes of General Medical Science.

LITERATURE CITED

1. Adams, M. H. 1959. Bacteriophages, p. 456. Intersciences Publishers, Inc., New York.
2. Avitabile, A., M. S. Carlomagno-Cerillo, R. Favre, and F. Blasi. 1972. Isolation of transducing bacteriophages for the histidine and isoleucine-valine operons in *Escherichia coli* K-12. J. Bacteriol. **112**:40–47.
3. Beckwith, J. R., and E. R. Signer. 1966. Transposition of the *lac* region of *E. coli*. I. Inversion of the *lac* operon and transduction of *lac* by φ80. J. Mol. Biol. **19**:254–265.
4. Fradkin, J. E., and D. G. Fraenkel. 1971. 2-keto-3-deoxygluconate 6-phosphate aldolase mutants of *Escherichia coli*. J. Bacteriol. **108**:1277–1283.
5. Fraenkel, D. G. 1967. Genetic mapping of mutations affecting phosphoglucose isomerase and fructose diphosphatase in *Escherichia coli*. J. Bacteriol. **93**:1582–1587.
6. Fraenkel, D. G., and S. Banerjee. 1971. A mutation increasing the amount of a constitutive enzyme in *Escherichia coli*, glucose 6-phosphate dehydrogenase. J. Mol. Biol. **56**:183–194.
7. Fraenkel, D. G., and S. Banerjee. 1972. Deletion mapping of *zwf*, the gene for a constitutive enzyme, glucose 6-phosphate dehydrogenase in *Escherichia coli*. Genetics **71**:481–489.
8. Fraenkel, D. G., and S. R. Levisohn. 1967. Glucose and gluconate metabolism in an *Escherichia coli* mutant lacking phosphoglucose isomerase. J. Bacteriol. **93**:1571–1578.
9. Gottesman, S., and J. R. 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.
10. Harris, A. W., D. W. A. Mount, C. R. Fuerst, and L. Siminovitch. 1967. Mutations in bacteriophage lambda affecting host cell lysis. Virology **32**:553–569.
11. Josephson, B. L., and D. G. Fraenkel. 1969. Transketolase mutants of *Escherichia coli*. J. Bacteriol. **100**:1289–1295.
12. Kelly, B. G., and M. G. Sunshine. 1967. Association of temperate phage P2 with the production of histidine negative segregants by *Escherichia coli*. Biochem. Biophys. Res. Commun. **28**:237–243.
13. Lennox, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190–206.
14. Lew, K. K., and J. R. Roth. 1971. Genetic approaches to determination of enzyme quaternary structure. Biochemistry **10**:204–207.
15. Low, K. B. 1972. *Escherichia coli* K-12 F-prime factors, old and new. Bacteriol. Rev. **36**:587–607.
16. Miller, J., K. Ippen, J. Scaife, and J. R. Beckwith. 1968. The promoter-operator region of the *lac* operon of *E. coli*. J. Mol. Biol. **38**:413–420.
17. Murray, M. L., and T. Klopotowski. 1968. Genetic map position of gluconate 6-phosphate dehydrogenase gene in *Salmonella typhimurium*. J. Bacteriol. **95**:1279–1282.
18. Peyru, G., and D. G. Fraenkel. 1968. Genetic mapping of loci for glucose-6-phosphate dehydrogenase, gluconate-6-phosphate dehydrogenase, and gluconate-6-phosphate dehydrase in *Escherichia coli*. J. Bacteriol. **95**:1272–1278.
19. Philipson, L., P. A. Albertsson, and G. Frick. 1960. The purification and concentration of viruses by aqueous polymer phage systems. Virology **11**:553–571.
20. Sato, K., Y. Nishimune, M. Sato, R. Numich, A. Matsushiro, H. Inokuchi, and H. Ozeki. 1968. Suppressor-sensitive mutants of coliphage φ80. Virology **34**:637–649.
21. Schlief, R., J. Greenblatt, and R. W. Davis. 1971. Dual control of arabinose genes on transducing phage λ *dara*. J. Mol. Biol. **59**:127–150.
22. Signer, E. R. 1964. Recombination between coliphages lambda and φ80. Virology **22**:650–651.
23. Signer, E. R. 1966. Interaction of prophage at the *att*80 site with the chromosome of *Escherichia coli*. J. Mol. Biol. **15**:243–255.
24. Signer, E. R., and J. R. Beckwith. 1966. Transposition of the *lac* region of *Escherichia coli*. III. The mechanism of attachment of bacteriophage φ80 to the bacterial chromosome. J. Mol. Biol. **22**:33–51.
25. Sistrom, W. R. 1958. On the physical state of the intracellularly accumulated substrates of β-galactosidase permease in *Escherichia coli*. Biochim. Biophys. Acta **29**:579–587.
26. Sunshine, M. G., and B. Kelly. 1971. Extent of host deletions associated with bacteriophage P2-mediated eduction. J. Bacteriol. **108**:695–704.
27. Sunshine, M. G. 1972. Dependence of eduction on P2 *int* product. Virology **47**:61–67.
28. Sussman, R., and F. Jacob. 1962. Sur un systeme de repression thermosensible chez le bacteriophage λ d'*Escherichia coli*. C. R. Acad. Sci. **254**: 1517–1520.
29. Sypirer, J., R. Thomas, and C. M. Radding. 1969. Hybrids of bacteriophages λ and φ80: a study of nonvegetative functions. Virology **37**:585–596.
30. Taylor, A. L., and C. D. Trotter. 1972. Linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **36**:504–524.
31. Voll, M. J. 1972. Derivation of an F-merogenote and a φ80 high-frequency transducing phage carrying the histidine operon of *Salmonella typhimurium*. J. Bacteriol. **109**:741–750.
332. Yamamoto, K. R., B. M. Alberts, R. Benzinger, L. Lahorne, and G. Treiber. 1970. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale virus purification. Virology **40**:734–744.