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Mapping of Insertion Mutations in *gnd* of *Escherichia coli* with Deletions Defining the Ends of the Gene

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A genetic map was prepared for *gnd*, the gene of *Escherichia coli* which encodes the metabolically regulated 6-phosphogluconate dehydrogenase. Direct selection methods were used for the isolation of mutants with deletions that define the respective ends of *gnd*. These selections depended on the availability of a defective lysogen in which *gnd* was present on a λ *h80* d*gnd his* prophage located at the *attφ80* region of the chromosome. Mutants with deletions entering *gnd* from the *his*-distal end were selected as Gnd⁻ TonB⁻ mutants. Mutants with *his*-proximal *gnd* deletions were selected as Gnd⁻, temperature-resistant mutants of a specially prepared stable lysogen. Gnd⁻ mutants were also isolated after mutagenesis with bacteriophage Mu *cts*61, and genetic tests were used to determine which mutants carry a Mu *cts*61 prophage in *gnd*. The deletion mutations were mapped against each other and against the insertion mutations through the use of F′ merodiploid strains. The insertion mutations mapped at seven distinct sites in *gnd*; three mapped under the deletions defining the *his*-proximal portion of the gene and three mapped with the *his*-distal deletions.

6-Phosphogluconate dehydrogenase (6PGD) (E.C. 1.1.1.44) catalyzes a key reaction of the hexose monophosphate shunt pathway of carbon metabolism. In *Escherichia coli* K-12, the amount of 6PGD increases about fourfold with increasing growth rate (25). The mechanism underlying this metabolic regulation is unknown, and it is not even known whether growth rate regulates transcription of the structural gene *gnd*. In our efforts to answer some of these questions, we have isolated two sets of *gnd* mutants and mapped the mutations. One set of mutants was induced by bacteriophage Mu. Mu has several special virtues as a mutagen. First, it integrates at random or nearly so in the *E. coli* chromosome (22), thereby causing a mutation in the gene in which it inserts. Thus, Mu is able to induce mutations in a relatively large number of sites, even within a single gene (6, 9). Second, Mu-induced mutations revert at very low frequencies (5, 12), which facilitates detection of recombination between them and other closely linked, non-reverting mutations, e.g., deletions. Third, Mu insertion mutations are strongly polar on transcription; transcription of a gene containing a Mu prophage proceeds into the prophage and terminates within it, with the result that the prophage-distal portion of the gene is not transcribed (8). Among other things, availability of strains carrying Mu insertion mutations of *gnd* might facilitate determination of the direction of transcription of *gnd* (8), purification of in vivo *gnd* mRNA (13), and isolation of *gnd* regulatory mutants after fusion of *lacZ* to *gnd* (2, 7). We also describe here the use of two direct selection methods for the isolation of a set of strains carrying deletions that enter *gnd* from each end of the gene. Both selections depended on the availability of a defective lysogen carrying the specialized transducing bacteriophage, λ *cI857 St68 h80* d*gnd his* (hereafter abbreviated λ *h80* d*gnd his*) (24), integrated at or near *attφ80* (R. E. Wolf, Jr., submitted for publication). In the work described here, the deletion strains were used to order the *gnd*::Mu mutations on the genetic map. In work to be reported elsewhere, we used the mutants to locate restriction endonuclease cleavage sites on the genetic map of *gnd* (M. S. Nasoff and R. E. Wolf, Jr., submitted for publication).

MATERIALS AND METHODS

Media and growth conditions. Minimal medium 63 (20) was supplemented with carbon source (4 mg/ml), thiamine hydrochloride (1 μg/ml), amino acids (25 μg/ml), and agar (2%). Sodium citrate (1.25 mg/ml) was added to minimal media to enhance growth of *tonB* mutants (11). The standard broth, BTYEX63, was medium 63 supplemented with 1% tryptone (Difco Laboratories) and 0.4% yeast extract (Difco). The standard rich plate was tryptone-yeast extract (TYE) agar (24). Antibiotic concentrations used were (per milliliter): streptomycin (Sm), 100 μg; rifampin (Rif), 50 μg; and nalidixic acid (Nal), 20 μg. LBC broth and plates (24) were used for the preparation and titration of lysates of bacteriophage Mu. SB broth (12) was used for preparations of lysates of Mu *cts*61. Gluconate

bromothymol blue indicator plates were prepared as described previously (26) and used to distinguish *gnd*$^+$ *edd* strains from *gnd edd* strains (see below).

Strains lysogenic for either λ *h80* d*gnd his* or Mu *cts61* are temperature sensitive and were grown at 30°C, as were strains carrying the F′(Ts) *trp*$^+$ *tonB*$^+$ *attφ80* episome. "Temperature-resistant" mutants were selected at 42°C; otherwise, growth was at 37°C.

Bacterial strains and scoring of *gnd*. Table 1 shows the bacterial strains of *E. coli* K-12 used in the present study. Strain RW211 was prepared as follows. A λ *h80* lysogen of strain RW181 was prepared, and a spontaneous Sm^r mutant was selected. Then a *thyA* derivative was selected, using the trimethoprim method described by Miller (15), and crossed with strain KL1699. Thy$^+$ Sm^r recombinants were selected, and those that coinherited *recA1* were identified by their sensitivity to UV light. Strain RW211 was one such recombinant that remained gluconate-negative, His$^-$, and resistant to λ *cI*. Preparation of other strains

TABLE 1. *Bacterial strains*

Strain	Genotype	Origin
W3110	F$^-$ prototroph	L. Soll
X′121	F$^-$ *galK pyrD his tyrA mtl thi str-125*	J. R. Beckwith
KL1699	Hfr *recA1*	B. Bachmann
8708	HfrH *trp*::Mu cIts61	A. Bukhari via J. Hillman
BU917	Hfr (KL16) *lacZ*::Mu	A. Bukhari via J. Hillman
X178	F′*trp*$^+$ *tonB*$^+$ *attφ80(φ80) colV colB/thi lacY14 Δ(trp-tonB)*	J. R. Beckwith
ER7008	F′(Ts) *trp*$^+$ *tonB*$^+$ *attφ80 lac*$^+$/*lac Δ(attφ80tonB-trp) recA*	J. R. Beckwith
RW181	F$^-$ *trpR lacZ*(Am) *trpA9605*(Am) *kdgR*^{c}*Δ(edd-zwf)22 Δ(sbcB-his-gnd-rfb)*	R. E. Wolf, Jr. (submitted for publication)
RW184	RW181(λ *cI857 St68 Δh80* d*gnd his*)2	R. E. Wolf, Jr. (submitted for publication)
RW184S	RW181(λ *cI857 St68 h80* d*gnd his*)	Single copy segregant of RW184
RW187-1, 2, 4, 5, 8–12, 14, 15, 18, 20	RW184S Δ(*tonB-attR-gnd*)	Ind$^+$ TonB$^-$ Gnd$^-$ His$^+$ mutants of RW184S; see text
RW187-3, 6, 7, 13, 16, 17, 19	RW184S Δ(*trpB-tonB-attR-gnd*)	Ind$^-$ TonB$^-$ Gnd$^-$ His$^+$ mutants of 184S; see text
RW199, RW200, RW201	RW184S Δ(*tonB-attR*)	"Stable" derivatives of 184S; see text
RW206	ER7008 (λ *cI857 h80*)	λ *cI857 h80* lysogen of ER7008
RW211	RW181 *recA1 str-125* (λ *h80*)	spontaneous Nal^r mutants
RW212-series	RW187-series Nal^r	See text
RW213-series	F′(Ts) *trp*$^+$*attφ80* (λ cI857 *h80*) *tonB*$^+$*lac*$^+$/ RW212(λ)	RW206 × RW212 on glucose + Nal, see text
RW214-series	F′(Ts) *his*$^+$ *Δ(gnd-attR-tonB) trp*/RW211	RW213 × RW211 on glucose + Trp + citrate + Sm
RW215-series	RW199 Δ(*imm-his-gnd*) *trpB*$^+$	"Temperature-resistant" mutants of the "stable" lysogen; see text
RW216-series	RW200 Δ(*imm-his-gnd*) *trpB*$^+$	"Temperature-resistant" mutants of the "stable" lysogen; see text
RW217-series	RW201 Δ(*imm-his-gnd*) *trpB*$^+$	"Temperature-resistant" mutants of the "stable" lysogen; see text
RW221-series	RW215 Nal^r (λ)	Spontaneous Nal^r mutant made lysogenic for λ
RW222-series	RW216 Nal^r (λ)	Spontaneous Nal^r mutant made lysogenic for λ
RW223-series	RW217 Nal^r (λ)	Spontaneous Nal^r mutant made lysogenic for λ
RW225	RW199 Nal^r	Spontaneous Nal^r mutant
RW231(λ)	RW181 *recA1 str-125* (λ)	R. E. Wolf, Jr. (submitted for publication)
RW231 (λ, Mu)	RW231(λ, Mu)	Mu lysogen of RW231(λ)
RW231 Rif^r (λ)	RW231 Rif^r (λ)	R. E. Wolf, Jr. (submitted for publication)
RW231 Rif^r (λ, Mu$^+$)	RW231 Rif^r (λ, Mu$^+$)	Mu lysogen of RW231 Rif^r (λ)
JC1, 2, 3b, 5b, 6a, 6b, 8a, 8b	RW225 *gnd* (Mu *cts61*)	Gnd$^-$ mutants induced by Mu *cts*; see text
JC18-1, etc.	Rif^r F′(Ts) *trp*$^+$ Δ(*tonB-attR*) *gnd his*$^+$/ RW231 Rif^r (λ, Mu$^+$)	JC1, etc. × RW231 Rif^r (λ, Mu) on glucose + Sm; see text

is described in the Results section. For genetic studies of *gnd*, strains must be blocked in the Entner-Doudoroff pathway of gluconate metabolism (10), e.g., by a mutation in *edd*. *gnd*$^+$ *edd* strains can use gluconic acid as sole carbon source and form green-colored colonies on gluconate BTB indicator plates, whereas *gnd edd* strains cannot use gluconate as sole carbon source and form white-colored colonies on the indicator plates. Nal^r mutants were selected as spontaneous mutants on TYE plates containing the antibiotic. λ lysogens were prepared by conventional methods.

Bacteriophages and preparation of lysates. Table 2 lists the bacteriophages used. A previously described plate method (24) was used to prepare lysates of φ80*v* at 2×10^{11} to 5×10^{11} plaque-forming units (PFU) per ml. Lysates of colicins V and B were prepared by UV light induction of strain X178 as described previously (24).

Lysates of Mu *cts*61 (12) were made by heat induction of strain 8708. Lysates of Mu$^+$ and Mu *c* were made by infection of liquid cultures (12). Remedies offered for the instability of Mu lysates (4, 13) were unsuccessful, and hence lysates were used within 1 week of preparation.

Mutagenesis by bacteriophage Mu and penicillin enrichment for *gnd* mutants. Plate mutagenesis was performed by the method of Boram and Abelson (3). Single drops of a freshly prepared lysate of Mu *cts*61 (ca. 10^9 phage) were spotted on a lawn of strain RW225 on LBC plates, and the plates were incubated at 28°C for 48 h. Turbid centers of plaques were picked and inoculated into glucose minimal medium or into BTYEX63 broth, and the cultures were grown to saturation at 32°C. After penicillin enrichment in gluconate minimal medium, carried out by the method of Miller (16), cultures were spread on gluconate BTB indicator plates. Gnd$^-$ colonies, which appeared after incubation at 32°C for 2 days, were cloned, and their phenotype was examined further. A reconstruction experiment showed that penicillin selection increased the frequency of Gnd$^-$ cells by 100-fold.

Phage methods. Strains lysogenic for Mu were identified by the presence of Mu immunity, using the "cross-streak" test described by Howe (12). A cross-streak test was also used to determine whether the tryptophan requirement of a given mutant was suppressible by φ80 p*suIII*, i.e., the presence of the amber mutation *trpA9605*.

Selection of deletion mutants. TonB$^-$ mutants were selected directly as described previously (24), and the mixture was spread on two gluconate BTB indicator plates and incubated for 2 days at 32°C. To identify TonB$^-$ mutants that have deletions that end in *gnd* or between *gnd* and *his*, Gnd$^-$ colonies were cloned and tested for histidine independence. To isolate "stable lysogens," e.g., strains RW199, RW200, and RW201, Gnd$^+$ TonB$^-$ mutants of strain RW184S, were cloned and streaked on gluconate BTB indicator plates at 42°C and scored for increased temperature sensitivity, with strain RW187-1 serving as control.

Mutants carrying *imm-his-gnd* deletions were selected as Gnd$^-$, temperature-resistant mutants of stable lysogens. A total of 10^8 cells cultured in LB broth at 32°C were spread on gluconate BTB indicator plates and incubated at 42°C for 2 days. Several Gnd$^-$ colonies from each selection were cloned and tested for a histidine requirement and the ability to use indole (Ind) in place of tryptophan (i.e., *trpB*$^+$).

Bacterial matings. Episome transfer experiments were carried out by mixing 0.5-ml donor and 0.5-ml recipient cultures, each in exponential growth and at 2×10^8 cells per ml, with 2 ml of fresh BTYEX63 broth in a 125-ml Erlenmeyer flask. After incubation at 32°C for 2 h without shaking, mating mixtures were suitably diluted and spread on the selective medium. When a large number of crosses were to be carried out, we reduced the volume of the mating mixture tenfold and used multicompartment dishes as described previously (24).

Measurement of 6PGD specific activity. Activity of 6PGD in sonic extracts was assayed as described previously (26). Protein was measured by the method of Lowry et al. (14). Specific activity is expressed in nanomoles of NADPH produced per minute per milligram of protein.

TABLE 2. *Bacteriophages*

Bacteriophage	Source
φ80 *v*	J. R. Beckwith
Mu *c*	A. Bukhari
Mu *cts*61	Heat induction of strain 8708 (12)
Mu-1	Spontaneous release from strain BU917
φ80 p*suIII*	L. Soll (17)
λ *c*I857 *h*80	S. Gottesman
λ *c*I	J. R. Beckwith

RESULTS AND DISCUSSION

We previously used *tonB* deletion analysis and marker rescue experiments to determine the location and order of bacterial genes in the φ80 d*gnd his* specialized transducing phage (24). For the work described here, we used strain RW184S which carries a single copy of λ *h*80 d*gnd his* (24) integrated at or near *att*φ80. λ *h*80 d*gnd his* is a λ-φ80 hyrid derivative of φ80 d*gnd his* (24). Figure 1 shows the genetic map of the prophage region of strain RW184S.

Selection of *gnd-tonB* deletion mutants. Mutants bearing deletions that enter *gnd* from the *his*-distal end (Fig. 1) and terminate within *gnd* or between *gnd* and *his* were isolated in two steps. First, *tonB* mutants were selected from 36 independent cultures of strain RW184S and spread on gluconate BTB indicator plates at 30°C. Several Gnd$^-$ TonB$^-$ colonies from each selection were cloned and then tested for histidine (His) independence and ability to use Ind. Thirteen independent His$^+$ Gnd$^-$ TonB$^-$ Ind$^+$ mutants and seven independent His$^+$ Gnd$^-$ TonB$^-$ Ind$^-$ mutants were recovered (strains RW187-1 to RW187-20).

The mechanism which served to integrate λ *h*80 d*gnd his* into the *E. coli* chromosome in the

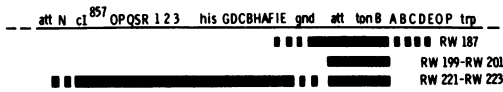

FIG. 1. *Prophage region of strain RW184S and deletion mutants. The order of genes imm-his-gnd-tonB-trp was determined by tonB deletion analysis as described in the text. The order of genes within the histidine operon and the location of E. coli DNA in the late region of φ80 is based on deletion analysis and marker rescue experiments of the parental phage, φ80d gnd his (24). imm is used to specify the region of λ between genes N and OP, which, when deleted, block host killing. att denotes the prophage termini; they may be either hybrid φ80 attachment sites or a translocatable element, depending on whether the phage integrated by int-mediated or site-specific recombination or by "replicon fusion" in the formation of strain RW184 (R. E. Wolf, Jr., submitted for publication). attR is between gnd and tonB. The tonB-gnd, tonB-attR, and imm-his-gnd deletions are indicated by the filled bars, with dashes to indicate the uncertainty of deletion endpoints.*

formation of strain RW184 is unknown; it may have been by *int*-mediated, site-specific recombination, or by replicon fusion (R. E. Wolf, Jr., submitted for publication). Regardless, the order of genes in the prophage region of strain RW184S as deduced by the above *tonB* deletion analysis is the same as that previously determined for the φ80 d*gnd his* lysogen, i.e., *imm-his-gnd-tonB-trp* (see Fig. 1).

Selection of *imm-his-gnd* deletion mutants. The direct selection of mutants with deletions that enter *gnd* from the *his*-proximal end has the following basis. Most cells in a culture of a λ cI857 lysogen die at high temperature (42°C) because phage functions lethal to the host are induced. The rare temperature-resistant survivors often bear deletions of the phage genes specifying or controlling the lethal functions (17, 19), e.g., λ *N* and λ *OP* (21), which are hereafter collectively abbreviated *imm*. Thus, by plating cultures of a λ *h*80 d*gnd his*-defective lysogen on gluconate BTB indicator plates at 42°C, one should be able to select directly mutants with deletions that cover *imm* and extend through *his* into *gnd* (see Fig. 1). However, strain RW184S is unsuitable for this selection, since it is unstable and segregates at a frequency of approximately 0.1% nonlysogenic cells whose phenotype, temperature resistance, His^- Gnd^-, is the same as that of the desired mutants. To circumvent this problem, we prepared derivatives of strain RW184S in which spontaneous loss of the prophage is blocked by a deletion that extends from *tonB* and removes "*attR*," the *trp*-proximal prophage terminus (Fig. 1). Gnd^+ $TonB^-$ mutants of strain RW184S were selected, and "stable lysogens" among them were identified by their property of yielding fewer Gnd^-, temperature-resistant clones than the parent when streaked on gluconate BTB indicator plates at 42°C.

Strains with *imm-his-gnd* deletions (see Fig. 1) were selected from 20 independent cultures of each of 3 independent stable lysogens (strains RW199, RW200, RW201) as described in Materials and Methods. The frequency of temperature-resistant clones was 10^{-6}, and 10% of these were Gnd^- His^- and sensitive to λ cI. To enrich further for mutants that carry deletions that end in *gnd* or between *gnd* and *trp*, we also tested for whether the tryptophan requirement of the various mutants was, like the parent strain, suppressible by φ80 p*suIII* (18). Suppressible deletion mutants were found in 53 of the selections. One from each was made Nal^r and lysogenic for λ, to facilitate subsequent mapping. The strains from the selections using strains RW199, RW200, RW201 are numbered in series RW221, RW222, RW223, respectively.

Mapping of the deletion mutations. The scheme for mapping the *gnd-tonB* deletions against the *imm-his-gnd* deletions is outlined in Fig. 2. We first transferred the prophage region of the strains of the RW187-series to an F′ episome by in vivo genetic recombination. This was done in two steps. First, the RW187 strains were made Nal^r, and an F′(Ts) trp^+ $tonB^+$ $att\phi80$(λ *h*80) episome was introduced into each by mating with strain RW206 and selecting Trp^+ Nal^r transconjugants. Then each partial diploid strain (RW212-series) was crossed with strain RW211, and His^+ Sm^r transconjugants were selected. The rationale for these crosses was that, since strain RW211 is *recA*, His^+ Sm^r transconjugants would most likely arise if an F′(Ts) trp^+ Δ(*tonB-gnd*) his^+ episome had been formed by recombination between the chromosome and the episome during growth of the given RW212 strain, and then transferred intact to strain RW211. His^+ Sm^r transconjugants were recovered from each cross at a frequency of approximately 1% per donor. Several transconjugants from each cross were cloned on the selective medium and tested for growth on gluconate as sole carbon source and for tryptophan independence. All were Gnd^- Trp^- His^+. One from each of the 20 crosses was retained for mapping purposes, the RW214 series strains.

We next prepared partial diploid strains with the *gnd-tonB* deletions on the exogenote and the *imm-his-gnd* deletions on the endogenote (Fig. 2). The strains of the RW214 series were crossed with the strains of the RW221, RW222, and RW223 series, and His^+ Nal^r transconjugants were selected by the "micro-mating" method described in Materials and Methods.

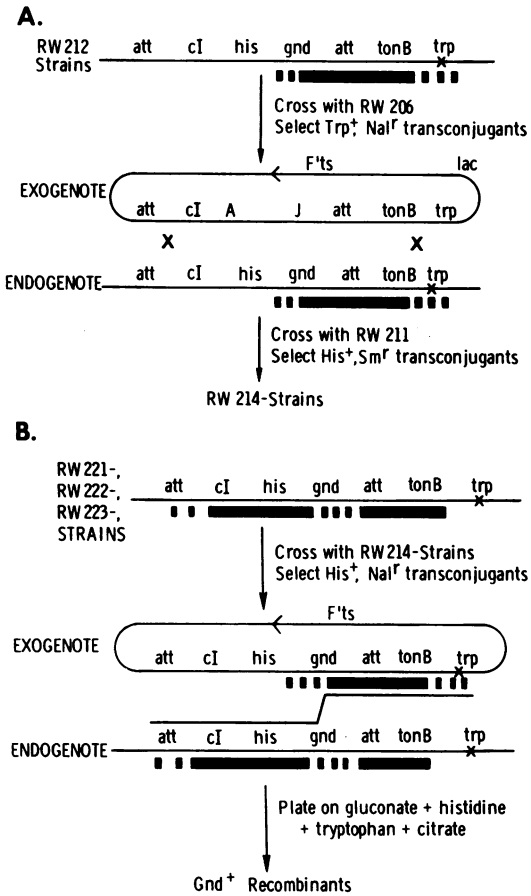

FIG. 2. *Scheme for deletion mapping. (A) Preparation of RW214 strains. (B) Crosses between imm-his-gnd deletion mutants and gnd-tonB deletion mutants.*

Transconjugants were cloned twice on the selective medium, and a single colony of each was inoculated into 0.2 ml of broth using multicompartment dishes. The cultures were grown at 30°C to saturation, and Gnd$^+$ recombinants were selected by spotting a portion of each culture (ca. 4×10^7 cells) onto gluconate minimal medium containing tryptophan, histidine, and citrate. Only eight of 1,060 partial diploid strains gave rise to Gnd$^+$ recombinants: the *imm-his-gnd* deletions of strains RW223-1 and RW223-3 were able to recombine with the *gnd-tonB* deletions of strains RW214-1, RW214-2, RW214-11, and RW214-12. The entire experiment was repeated with the same result. To make sure that no *gnd-tonB* deletions able to recombine with the deletions carried by strains RW223-1 and RW223-3 were missed in the micro-method, two colonies of each of the respective 40 merodiploids were again inoculated into broth, grown for approximately 30 generations, and plated for Gnd$^+$ recombinants. The eight merodiploids identified above again gave rise to Gnd$^+$ recombinants at a frequency of approximately 10^{-5}, whereas the deletions in the remaining strains did not recombine ($<5 \times 10^{-9}$).

The genetic map based on these crosses (Fig. 3) shows that the *imm-his-gnd* deletions of strains RW223-1 and RW223-3 define the *his*-proximal end of *gnd*, and that the *gnd-tonB* deletions of strains RW214-1, RW214-2, RW214-11, and RW214-12 define the *his*-distal end of the gene; i.e., no opposing deletions were found to overlap.

Considering the relatively small distance between *gnd* and *trpB* in the stable lysogens (1) (Fig. 1), it was somewhat unexpected that only two of 53 *imm-his-gnd* deletions terminated in *gnd*. This frequency suggests that formation of deletions extending from *imm* to *his* is nonrandom. Indeed, a "hot-spot" for deletion-formation has been identified near *gnd*, on the *his*-distal side (R. E. Wolf, Jr., submitted for publication), and thus most of the strains selected as temperature-resistant and Gnd$^-$ would be expected to bear deletions that cover *gnd* completely.

The endpoints of the *gnd-tonB* deletions appear to be random. The distance between *his* and *gnd* is sufficient to accommodate about four average-sized genes (1). Thus, if deletion endpoints were random, one would predict that about one of five *his*$^+$ Δ(*gnd-tonB*) deletions would terminate in *gnd*, and indeed, four of 20 RW187 strains were found to carry such deletions.

The reason for the low frequency at which positive recombination occurred (10^{-5} to 10^{-6}) is not clear. Either the respective ends of the deletions are very close, or the limiting step in recombination is not the crossover between the ends of the deletions, but rather the synapsis of the recombining chromosomes, because of the limited homology on either side of *gnd*.

Isolation and initial characterization of gnd mutants induced by Mu. Strain RW225 was treated with Mu and Gnd$^-$ mutants selected as described in Materials and Methods. Strain RW225, a "stable lysogen," was chosen for Mu

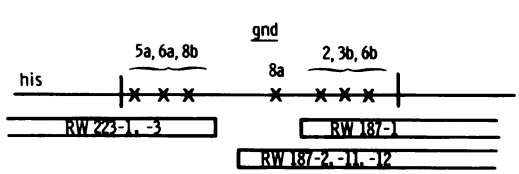

FIG. 3. *Genetic map of gnd. The order of the seven of gnd::Mu insertion mutations (×) (see Table 4) within a region noted by parentheses has not been determined.*

mutagenesis, rather than an *edd* mutant with *gnd* at its normal chromosomal position, because of the ease with which episomes carrying the prophage region could be prepared and used for subsequent mapping. The overall frequency of recovery of colonies with the phenotype of Gnd$^-$ mutants was approximately 3×10^{-4}. Several Gnd$^-$ colonies from each of 26 selections were cloned and tested further. The initial screening was for mutants which were: (i) unable to grow on gluconate (Gna) minimal media containing tryptophan and citrate; (ii) able to use both arabinose (Ara) and glucose (Glu) as carbon sources on minimal media containing indole and citrate; (iii) Mu lysogens, by the criterion of being resistant to Mu *c* in a cross-streak test (see Materials and Methods). From these tests, 12 Mu lysogens were identified which were Gna$^-$ Ara$^+$ Glu$^+$ His$^+$ Ind$^+$, the phenotype of the desired *gnd* mutants. Eleven of these failed to revert ($<10^{-10}$), as expected for Mu-induced mutants (5). Most of the remaining tested Gnd$^-$ colonies were either not lysogenic for Mu *cts*61 or were His$^-$; the Gnd$^-$ His$^-$ mutants probably carry deletions induced by the above-mentioned nearby hot spot for deletion formation or by Mu (21). The frequency of recovery of Mu-induced Gnd$^-$ mutants was estimated to be only slightly greater than the appearance of spontaneous *gnd* mutants in cultures of strain RW225 (data not shown). This is probably due to a combination of two factors: (i) the high frequency of deletion formation, and (ii) the fact that *gnd* is transcribed at a relatively high frequency, 0.5% of cell protein being 6PGD (26), and mutants carrying Mu insertion mutations in such genes are recovered at a reduced frequency (9).

The inability of the Mu-induced mutants of strain RW225 to use gluconate as carbon source might be due to a lack of the *gnd* gene product, 6PGD, or to a defect in gluconate transport or gluconate kinase (10). Accordingly, we assayed each mutant for 6PGD activity and found that none of the mutants had any detectable activity (data not shown).

Zygotic induction test for the presence of a Mu prophage in gnd. We used strains with F′ episomes that carry the prophage region of the various Mu-induced Gnd$^-$ mutants and a zygotic induction test to determine whether the *gnd* mutation was due to the presence of a Mu prophage inserted into the gene (4). Availability of the strains carrying these episomes also facilitated the mapping of the mutations (see below). Figure 4A shows the scheme for preparation of these strains. First an F′(Ts) *trp*$^+$ *tonB*$^+$ *att*ϕ*80*(λ *c*I857 *h*80) episome was introduced into each mutant by mating with strain RW206 and

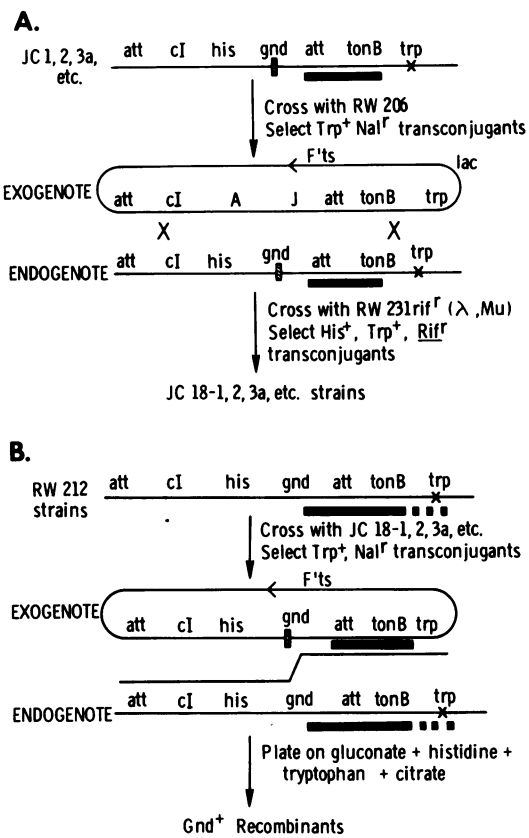

FIG. 4. *Scheme for mapping gnd::Mu mutations. (A) Preparation of strains of JC18-series. (B) Crosses of gnd::Mu mutants with deletion mutants of the RW212 strains. Mapping of imm-his-gnd deletions followed a similar protocol. Mu insertion mutations are indicated by vertical, hatched bars; deletions are indicated by filled bars.*

selecting Trp$^+$ Nal^r transconjugants on glucose minimal medium containing nalidixic acid. A transconjugant from each cross was cloned, grown in broth, and mated with strain RW231 (λ, Mu). His$^+$ Trp$^+$ Sm transconjugants were selected from each of these crosses by plating on glucose minimal medium containing streptomycin. We wanted to obtain F′ episomes which carry not only the *his-gnd* region of the various mutants but also the *trp*$^+$ marker of the parental episome because the presence of *trp*$^+$ would facilitate subsequent mapping studies. His$^+$ Trp$^+$ Sm^r transconjugants were recovered from each cross at a frequency of 10^{-6} to 10^{-7}. These strains are numbered JC18-1, -2, -3a, -3b, etc., in correspondence with the original mutants. All were shown to be Gnd$^-$.

Episomes of the strains of the JC18 series were tested for carrying Mu by mating with

strain RW231 Rif^r (λ, Mu) and strain RW231 Rif^r(λ). Dilutions of each mating mixture were plated on glucose minimal media containing rifampin to select for His$^+$ Trp$^+$ Rif^r transconjugants. Results are presented in Table 3. Strains JC18-2, 3b, 5a, 6a, 6b, 8a, and 8b gave rise to zygotic induction, which shows that Mu is on the episome of these strains, probably in *gnd*. Strains JC18-1, 18-3a, 18-6c, and 18-7 did not show zygotic induction. Therefore, the episomes of these strains do not carry Mu and strains JC1, 3a, 6c, and 7 probably carry deletions or insertions in *gnd*, with the Mu prophage located elsewhere.

Deletion mapping of the *gnd*::Mu mutations. Mu insertion mutations map as point mutations. We mapped the seven *gnd*::Mu mutations against the deletions which were previously determined to end within *gnd* by selecting Gnd$^+$ recombinants from cultures of merodiploids in which the *gnd*::Mu mutations are on the exogenote and the deletions are on the endogenote (see Fig. 4B). This mapping method depends on the fact that neither *gnd* mutation in the merodiploid reverts; thus, under the conditions used, recombination frequencies as low as 10^{-8} were detectable.

Strains JC18-2, -3b, -5a, -6a, -6b, -8a, and -8b were crossed with Mu lysogenic derivatives of strains RW212-1, RW212-2, RW212-11, RW212-12, RW223-1, and RW223-3, and Trp$^+$ Nal^r transconjugants were selected. The resulting merodiploid strains were cloned, grown in broth for approximately 30 generations, and plated on to select Gnd$^+$ recombinants. Results are shown in Table 4. The frequency of positive recombination was low, approximately 10^{-5} to 10^{-6}. A genetic map of *gnd* (Fig. 3) can be deduced from the data of Table 4. It shows that the *gnd*::Mu mutations map in one of three regions as defined by the deletions.

We next examined the possibility that the *gnd*::Mu mutations might be further partitioned on the genetic map by their ability to recombine with other deletions in the RW212, RW221, RW222, or RW223 series of strains, a possibility since some of these strains might carry deletions that end in *gnd* but which were not identified because they overlap all other deletions. The F′ *gnd*::Mu episomes of the JC18 strains were introduced into the strains of the RW212-, RW221-, RW222-, and RW223-series by the micro-mating method and selection of Trp$^+$ Nal^r transconjugants. The resulting merodiploids were cloned and grown to saturation in broth, and Gnd$^+$ recombinants were selected as described in Materials and Methods. Gnd$^+$ recombinants were recovered from each of the merodiploid strains which had previously been shown to recombine, but no additional positive recombination was observed, with a limit of detectability of approximately 4×10^{-7} (data not shown).

To determine whether the *gnd*::Mu mutations which map in a given region occupy the same or different sites, pairwise crosses were made between them, and Gnd$^+$ recombinants were selected. The F′ *gnd*::Mu episomes of the JC18 series strains were introduced into mutants JC2, -3b, -5a, -6a, -6b, -8a, and -8b by mating and selection of Trp$^+$ Nal^r transconjugants as described above. Then merodiploids were cloned, and Gnd$^+$ recombinants were selected. All pairs of *gnd*::Mu mutations yielded Gnd$^+$ recombinants except for the "self-crosses." Thus, the *gnd*::Mu mutations map at seven distinct sites in *gnd*: three lying under the deletions defining the more *his*-proximal portion of the gene, and three lying under the deletions defining the more *his*-distal portion. This distribution of mutations accords with the previous observation that Mu insertion mutations within a given gene occur randomly or nearly so, even when the overall frequency of Mu-induced mutations in the gene is reduced by a relatively high rate of transcription of the gene (9). It also suggests that the endpoints of the deletions that terminate within *gnd* are quite close.

In work to be reported elsewhere (M. S. Nasoff and R. E. Wolf, Jr., submitted for publication), we prepared by in vitro recombination hybrid plasmids which carry *gnd* and subgene portions of it. We used these plasmids and mutants described here to correlate the restriction and genetic maps of *gnd* and to determine that the *gnd* promoter lies at the *his*-distal end of the gene. The availability of strains with *gnd*::Mu *cts*61 mutations mapped with respect to the promoter should facilitate the construction of *gnd-lac* fusion strains (2), and thus the isolation of mutants defective in growth rate-dependent regulation of 6PGD synthesis (2, 7).

TABLE 3. *Zygotic induction by transfer of F′ gnd*::*Mu episomes*

Donor	Trp$^+$ Rif^r Transconjugants	
	RW231 Rif^r (λ, Mu)	RW231 Rif^r (λ)
JC18-1	750	750
JC18-2	1,000	20
JC18-3a	1,000	1,000
JC18-3b	1,000	10
JC18-5a	1,000	25
JC18-6a	750	30
JC18-6b	1,000	20
JC18-6c	750	500
JC18-7	400	400
JC18-8a	1,000	12
JC18-8b	1,000	15

TABLE 4. *Deletion mapping of gnd::Mu mutations*

Donor (exogenote)	Recipient (exogenote)					
	RW212-1	RW212-2	RW212-11	RW212-12	RW223-1	RW223-3
JC18-2	0	0	0	0	+	+
JC18-3a	+	+	+	+	0	0
JC18-3b	0	0	0	0	+	+
JC18-5b	+	+	+	+	0	0
JC18-6a	+	+	+	+	0	0
JC18-6b	0	0	0	0	+	+
JC18-8a	+	0	0	0	+	+
JC18-8b	+	+	+	+	0	0

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