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Growth-Rate-Dependent Expression and Cloning of *gnd* Alleles from Natural Isolates of *Escherichia coli*

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6-Phosphogluconate dehydrogenase (6PGD), encoded by *gnd*, is highly polymorphic among isolates of *Escherichia coli* from natural populations. As a means of characterizing the growth-rate-dependent regulation of the level of 6PGD, five *gnd* alleles, including the *E. coli* B/r allele, were crossed into *E. coli* K-12 with bacteriophage P1. In each of the isogenic strains, the level of 6PGD was two- to threefold higher in cells grown on glucose than in cells grown on acetate. The level of enzyme activity in the acetate-grown cells varied about sixfold within the set of isogenic strains. The physiological importance of these differences in enzyme level is discussed. The *gnd* gene was cloned from five *E. coli* strains and *Salmonella typhimurium* LT-2 and mapped with twelve restriction endonucleases. *gnd* was located and oriented on the chromosomal DNAs. The restriction maps of the genes were aligned at conserved restriction sites, and the relative divergence of the genes was estimated from restriction site polymorphisms. The *E. coli gnd* genes differed from the *S. typhimurium* gene by about 11%. Most of the *E. coli* genes differed from one another by less than 5%, but one allele differed from the others by about 10%. Only the *gnd* gene from *E. coli* K-12 had an IS5 element located nearby.

In *Escherichia coli* K-12, the expression of *gnd* is coupled with the cellular growth rate such that the level of 6-phosphogluconate dehydrogenase (6PGD) is proportional to the growth rate during growth in minimal media (16, 37). The properties of *gnd-lac* operon and protein fusion strains have suggested that this growth-rate-dependent regulation occurs at a posttranscriptional step and requires a site of negative control that lies within the 6PGD-coding sequence, between codons 48 and 118 (2, 3).

As an approach toward identifying the sequences in *gnd* that are important to the regulation, we have taken advantage of the fact that the *gnd* locus is highly polymorphic among *E. coli* strains isolated from natural populations (22, 32). The overall strategy was to introduce *gnd* genes from these natural isolates into a standard *E. coli* K-12 strain, determine the growth rate dependence of 6PGD level in each strain, clone the respective *gnd* genes, and determine the DNA sequences of the region between the promoter and codon 124 of the structural gene.

In the work presented here, we describe the preparation of a set of six isogenic strains differing only at the *gnd* locus. The strains described here and their properties have provided a comparative basis for DNA sequence analysis, which is presented in the companion paper (4).

MATERIALS AND METHODS

Media and growth conditions. The media used were LB broth (23) for routine bacterial subculture; TB broth and top agar (7) for the propagation of strains during manipulations with bacteriophage λ; TBYCM broth (23) for the preparation of lysates of phage P1; F-top and H-top agars (23) for generalized transductions; and YT agar plates and broth (21) for the isolation, cloning, and propagation of M13 phage and M13 DNA. Antibiotics included ampicillin (40 μg/ml), chloramphenicol (15 μg/ml for selection of P1 *cml clr100* lysogens, 25 μg/ml for selection of λYL460 lysogens, and 175 μg/ml for plasmid amplification), and tetracycline (25 μg/ml). Minimal medium 63 (23) with appropriate supplements was used for genetic selections. MOPS (morpholinepropanesulfonic acid) medium was used in growth rate experiments as previously described (37). Growth rates are expressed as the specific growth rate constant (*k* [37]). The Lac phenotypes were determined on lactose MacConkey indicator medium or by the addition of 5-bromo-4-chloro-3-indolyl-β-D-galactoside (XG) to solid medium at a final concentration of 40 μg/ml. Strains lysogenic for bacteriophage P1 *cml clr100* were cultured at 30°C. All other strains were grown at 37°C.

Bacterial strains. Table 1 shows the *E. coli* K-12 strains used in this study.

In *E. coli*, gluconate can be metabolized via 6PGD and the nonoxidative branch of the hexose monophosphate shunt or the Entner-Doudoroff pathway (17). The Gnd phenotype is conveniently revealed in an *edd* mutant, in which the Entner-Doudoroff pathway is abolished. Gluconate BTB indicator medium (38) was used to distinguish Edd$^-$ Gnd$^-$, Edd$^-$ Gnd$^+$, and Edd$^+$ bacteria (35).

The growth rate control of *gnd* expression was previously studied in the *E. coli* K-12 strain W3110 (2, 3, 37). To extend this work, the various *gnd* alleles were crossed into a strain W3110 derivative with phage P1. The P1 donors are shown in Table 2. The recipient was strain GB355 which carries *his*::Tn*10* and has a *gnd-lac* operon fusion. It was made by growing P1 on strain HB355 and transducing W3110 to tetracycline resistance. Strain GB355 was a His$^-$ transductant that coinherited the fusion. The presence of the fusion was scored on glucose (2%) minimal medium containing XG.

Strain GB23152 was the recipient in the cloning of the *gnd* alleles from the non-*E. coli* K-12 strains. This strain transforms well and carries *hsdR recA* Δ*gnd* Δ*edd*. It was derived from strain RW231. Since strain RW231 lyses in the presence of calcium chloride concentrations routinely used in bacterial transformation (unpublished results), the calcium chloride-resistant mutant, RW231Ca, was selected by sequential subculturing in the presence of 5, 10, 20, and 28 mM

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TABLE 1. Bacterial strains

Strain	Genotype and phenotype[a]	Source or reference
E. coli K-12		
GB355	W3110 *gnd-128*::ΔMu cts d1(Ap^r Lac)::λp1 (209) (Lac$^+$) *his*::Tn*10*	This study
GB3079	NF3079-1 *recA3*	P1 (N100) × NF3079-1
GB23152	RW231Ca *hsdR*	This study
GB310558	W3110 [*gnd*(558)][b]	This study
GB310567	W3110 [*gnd*(567)]	This study
GB310740	W3110 [*gnd*(740)]	This study
GB310745	W3110 [*gnd*(745)]	This study
GB310B/r	W3110 [*gnd*(B/r)]	This study
HB101	F$^-$ *hsdS20*(r_B^- m_B^-) *recA13 ara-14 proA2 lacY1 galK2 rpsL20*(Str^r) *xyl-5 mtl-1 supE44*	C. L. Keeler (10)
HB352	W3110 F$^-$ Δ(*edd-zwf*)*22 zeb-1*::Tn*10* Δ(*argF-lac*)U169 *gnd-128*::ΔMu cts d1 (Ap^r Lac)::λ p1(209) (Lac$^+$)	(2)
HB355	W3110 Δ(*argF-lac*)U169 *gnd-128*::ΔMu cts d1(Ap^r Lac)::λp1 (209) (Lac$^+$) *his*::Tn*10*	(2)
JM109	*recA1 endA1 gyrA96 thi hsdR17 supE44 relA1* Δ(*lac-proA,B*)/ *F' traD36 proA*$^+$*B*$^+$ *lacI*^{q} Δ(*lacZ*)M15	S. Bingham (39)
KK2186	Δ(*lac-pro*) *supE thi endA sbcB15 rpsL20 hsdR4*/F' *traD36 proA*$^+$*B*$^+$ *lacI*^{q} Δ(*lacZ*)M15	O. Karlström
N100	W3102 *recA3 galK* Str^r	J. Hays
NF3079	F$^-$ *araD139* Δ(*araABOIC-leu*)*7679 galU galK* Δ(*lac*)*X74 rpsL*(Str^r) *thi dam-3*	N. Fiil
NF3079-1	NF3079 *srl*::Tn*10*	P1(TS001) × NF3079
RW231	W3110 *trpR lacZ*(Am) *trpA9605 kdgR*^{c} Δ(*edd-zwf*)*22* Δ(*sbcB-his-gnd-rfb*) *recA rpsL20*	(35)
RW231Ca	RW231 Ca^r	This study
TS001	*galK2 srl*::Tn*10 rpsL*(Str^r)	T. Smith
W3110	F$^-$ prototroph	This laboratory
S. typhimurium LT-2		
SB3436	*hisT1504*	P. Hartman (34)

[a] The notations used are those of Bachmann (1). Str^r, Streptomycin resistant; Ca^r, calcium resistant.
[b] Brackets indicate that *gnd* was introduced from another *E. coli* strain by P1 transduction.

calcium chloride. The optimal calcium chloride concentration for plasmid transformation of this strain was 70 mM. An *hsdR* derivative of RW231Ca was isolated with λYL460 and selection for chloramphenicol-resistant (Cm^r) lysogens (12). λYL460 has a Tn*9* insertion and was obtained from J. L. Rosner. Several putative *hsdR* mutants were heat cured of the λYL460 prophage. An *hsdR* mutant that still carries *hsdM*$^+$, strain GB23152, was identified by its ability to modify λ *vir* phage.

Bacteriophages and phage methods. Phage P1 *cml clr100* (P1) and λYL460 lysates were prepared by heat induction of lysogens as described by Miller (23). P1 *vir*, λ *c*I, and λ *vir* were grown by the plate method (23). Generalized transductions with P1 and P1 *vir* lysates were performed as described by Miller (23). Phage λ specialized transductions were performed as previously described (23). All manipulations of bacteriophage M13 were performed according to Messing (21). Other phages are shown in Table 3.

Recombinant DNA techniques. Plasmid DNA was isolated by the alkaline lysis method (9) and further purified by treatment with RNase A, proteinase K, phenol extraction, and precipitation at 0°C from 0.5 M NaCl–10% (wt/vol) polyethylene glycol (PEG 8000; J. T. Baker Chemical Co., Phillipsburg, N.J.). Chromosomal DNAs were prepared as described by Berman et al. (7). Restriction enzymes (Bethesda Research Laboratories, Inc., Gaithersburg, Md. or New England BioLabs, Inc., Beverly, Mass.) were used according to the recommendations of the supplier. Plasmid DNAs were isolated from *recA* strain GB23152 or *dam* strain GB3079 when *Bcl*I restriction sites were analyzed.

The *E. coli* K-12 *gnd* [*gnd*(K-12)] DNA sequence (26) shows *Kpn*I and *Pvu*II fragments of 411 and 432 base pairs, respectively. These sites are located 5′ to a carboxy-terminal *Pst*I site. The six newly cloned *gnd* loci showed similar restriction patterns for these sites. Replicative-form (RF) DNAs of phages M13mp18 and M13mp19 were isolated by the alkaline lysis method (9) and purified by CsCl density gradient centrifugation (20). Restriction fragments were subcloned into M13 RF DNA by standard methods (21). Strain JM109 was used to propagate recombinant M13 phages. In some cases, restriction fragments were first purified by agarose gel electrophoresis (6). C-tests (5, 21) with M13 phage DNA carrying the sense (mHB562-9) or antisense (mKD14) strand of the *gnd*(K-12) gene were used for screening the recombinant phages and determining which strand was carried by the recombinant phages. The recombinant M13 phages are shown in Table 3. Other standard recombinant DNA methods were used (20).

Measurement of enzyme specific activity. The activity of 6PGD in whole-cell sonicated extracts was measured spectrophotometrically as described by Wolf et al. (37); units are nanomoles of NADPH formed per minute at 25°C. The protein concentration was determined by the method of Bradford (11), with immunoglobulin G as the standard. Absorbances for enzyme activity were determined with a Gilford model 250 spectrophotometer, equipped with a constant-temperature circulating water bath. Protein determinations were made with a Gilford Stasar II spectrophotometer. Duplicate enzyme and protein assays were done on two samples from each culture.

TABLE 2. Sources of *gnd* alleles used to prepare isogenic *E. coli* K-12 strains

Strain	Original designation[a]	Host[b]	Mobility class[c]	*gnd* allele[d]	Reference
DD558	RM202F	Ape	F2	*gnd*(558)	22, 13
DD567	RM200Q	Pig	S8	*gnd*(567)	13
DD740	RM43A	Human	W^+	*gnd*(740)	13
DD745	RM72B	Gorilla	S4	*gnd*(745)	13
NC3 (B/r)	NA[e]	Unknown	F2	*gnd*(B/r)	33
W3110	NA	Human	K-12 (W^+)	*gnd*(K-12)	

[a] Strain designations prefixed by RM are from the Roger Milkman collection and were obtained from D. Hartl and D. Dykhuizen. Strains RM200Q and RM202F are in groups I and II, respectively, in the *E. coli* reference collection (28). The other strains have not been classified.

[b] Original source.

[c] Electrophoretic class. The order of increasing electrophoretic mobility is S8, S4, W^+, and F2.

[d] The assigned *gnd* alleles as used in the text.

[e] NA, Not applicable.

Cellulose acetate electrophoresis of whole-cell extracts. Whole-cell sonic extracts were prepared from cells grown in LB broth as described by Wolf et al. (37) and separated by cellulose acetate electrophoresis essentially as described by Eicher and Washburn (14). Electrophoresis with cellulose acetate matrices (Helena Laboratories, Beaumont, Tex.) was performed at 260 V for 25 min. 6PGD enzyme activity was detected by overlaying the matrix with 4.0 ml of a staining solution containing 25 mM Tris hydrochloride (pH 8.5), 2.5 mM $MgCl_2$, 0.5 mM NADP, 1.25 mM 6-phosphogluconate, 0.05 mg of phenazine methosulfate per ml, 0.25 mg of [3-(4,5-dimethylthiazolyl-2)]-2,5-diphenyltetrazolium bromide per ml in 0.3% agarose. The staining was done in the absence of light and generally was complete within 10 min.

RESULTS

Preparation of isogenic *E. coli* K-12 strains differing only at *gnd*. Table 2 shows the original *E. coli* isolates from which the different alleles were obtained and the electrophoretic mobility classes of the respective 6PGD allozymes. To compare the growth-rate-dependent regulation of the various *gnd* alleles, we constructed a set of isogenic strains differing only at the *gnd* locus. This was accomplished by using bacteriophage P1-mediated transduction to introduce the different *gnd* alleles into the *E. coli* W3110 strain which was used in earlier studies (37). Multiple rounds of transduction were used to minimize the amount of non-*E. coli* K-12 DNA at the *gnd* locus, and after each round, the presence of the correct 6PGD allozyme was verified by cellulose acetate electrophoresis. First, bacteriophage P1 *cml clr100* lysates were prepared on the various natural isolates or *E. coli* B/r strain NC3, by heat induction of lysogens. Gna^+ transductants of strain HB352 were selected on gluconate minimal medium containing XG. As expected, both Edd^+ and Gnd^+ transductants were recovered; they could be distinguished by their Lac phenotype. The Edd^+ transductants retained the *gnd-lac* operon fusion and were Lac^+, whereas the Gnd^+ transductants lost the fusion and hence were Lac^-. Gnd^+ Lac^- transductants were obtained from each of the first rounds of transduction and were scored for tetracycline resistance (Tc^r), λ and chloramphenicol sensitivity, and the electrophoretic mobility of 6PGD.

Unexpectedly, all the gnd^+ transductants with strains DD558, DD740, and DD745 as donors were insensitive to λ *c*I. Since the Edd^+ transductants in these same crosses were all sensitive to λ *vir*, the λ resistance phenotype of the Gnd^+ transductants was probably linked to *gnd*. To make the various strains more identical and to obtain λ *vir*-sensitive derivatives of these strains, primary transductants carrying the aforementioned alleles were infected with phage λYL460 and Cm^r transductants were selected. The Cm^r transductants were λ *vir* sensitive.

Generalized transducing lysates were prepared on the first-round transductants or their λ-sensitive derivatives and used to transduce strain HB352 to growth on gluconate minimal medium. The second-round transductants were Gnd^+, Lac^-, Tc^r, λ *c*I and chloramphenicol sensitive, and had the expected 6PGD allozyme. In the final round of

TABLE 3. Bacteriophages

Phage	Relevant genotype[a]	Source or reference
mGB558KE2	*gnd'*(558) (antisense)	1-kb *Eco*RI-*Kpn*I fragment from pGB310558 in M13mp18
mGB558KB1	*gnd'*(558) (sense)	1.3-kb *Bam*HI-*Kpn*I fragment from pGB310558 in M13mp18
mGB567KH1	*gnd'*(567) (antisense)	0.86-kb *Hin*dIII-*Kpn*I fragment of pGB310567, resulting from a partial *Kpn*I digestion in M13mp19
mGB567KH3	*gnd'*(567) (antisense)	0.72-kb *Hin*dIII-*Kpn*I fragment from pGB310567 in M13mp19
mGB567DF3	*gnd'*(567) (sense)	1.3-kb *Eco*RI-*Bgl*II (made flush with Klenow fragment) fragment from mGB567KH3 RF in M13mp18
mGB740KE6	*gnd'*(740) (antisense)	2.7-kb *Eco*RI-*Kpn*I fragment from pGB310740 in M13mp18
mVL740RVK	*gnd'*(740) (sense)	0.49-kb *Eco*RV-*Kpn*I fragment from pGB310740 in *Hin*cII- and *Kpn*I-cut M13mp18
mGB745KH5	*gnd'*(745) (antisense)	0.62-kb *Hin*dIII-*Kpn*I fragment from pGB310745 in M13mp19
mGB745DF3	*gnd'*(745) (sense)	0.62-kb *Hin*dIII-*Kpn*I fragment from pGB310745 in M13mp18 with a spontaneous deletion of a portion of *gnd*(745)*p*
mGBB/rC2	*gnd'*(B/r) (antisense)	2.2-kb *Hin*dIII-*Kpn*I fragment from pGB310B/r in M13mp19
mVLB/rRVK	*gnd'*(B/r) (sense)	0.49-kb *Eco*RV-*Kpn*I fragment from pGB310B/r in *Hin*cII- and *Kpn*I-cut M13mp18
mGB3436KE1	*gnd'*(3436) (antisense)	0.9-kb *Eco*RI-*Kpn*I fragment from pGB3436 in M13mp18
mGB3436H13	*gnd'*(3436) (sense)	0.76-kb *Hin*dIII fragment from pGB3436 in M13mp18
M13mp18		S. Bingham; (27)
M13mp19		S. Bingham; (27)
mKD14	*gnd'*(K-12) (antisense)	1.13-kb *Bcl*I-*Pst*I fragment of *gnd*(K-12) in M13mp8 (25)
mHB562-9	*gnd'*(K-12) (sense)	1.25-kb fragment of *gnd*(K-12) in M13mp9 (25)

[a] Sense and antisense indicate whether the transcribed or opposite strand of *gnd* is carried by the phage, respectively.

TABLE 4. Growth rate dependence of 6PGD in isogenic *E. coli* K-12 strains differing only at *gnd*[a]

Strain	6PGD mobility class	*k*		Sp act[b]		Induction ratio
		Acetate	Glucose	Acetate	Glucose	
W3110 (K-12)	W^+	0.19	0.71	73 ± 5	206 ± 24	2.8
GB310745	S4	0.14	0.59	67 ± 3	229 ± 10	3.4
GB310740	W^+	0.17	0.61	40 ± 7	97 ± 15	2.4
GB310B/r	F2	0.17	0.65	36 ± 3	77 ± 11	2.1
GB310567	S8	0.21	0.67	30 ± 2	53 ± 4	1.8
GB310558	F2	0.18	0.58	12 ± 2	21 ± 3	1.8

[a] Cultures were grown in acetate and glucose MOPS medium and assayed as described in Materials and Methods.
[b] Values given consist of the mean ± standard deviation.

transduction, phage P1 *vir* was used to introduce the respective gnd^+ alleles into the edd^+ strain GB355 [*his*::Tn*10* Φ (*gnd-lac*)] by selecting His^+ transductants. His^+ Lac^- transductants, i.e., his^+ gnd^+, were tested for their *gnd* allele by cellulose acetate electrophoresis.

Growth-rate-dependent regulation of 6PGD level in isogenic *E. coli* K-12 strains. The specific activity of 6PGD was measured in the isogenic strains grown in acetate and glucose MOPS minimal media. The basal level varied over a sixfold range (Table 4). The growth rate induction in strain W3110 was about threefold, as previously observed (16, 37). Expression of the other *gnd* alleles was also growth rate regulated, although the induction ratios varied from 1.8- to 3.4-fold.

Molecular cloning of *gnd* alleles. Chromosomal DNA was isolated from each of the *E. coli* K-12 strains with *gnd*(558), *gnd*(567), *gnd*(740), *gnd*(745), and *gnd*(B/r) and from the *Salmonella typhimurium* LT-2 strain SB3436. The DNAs were digested with *Hin*dIII or *Eco*RI and ligated to similarly cleaved plasmid pBR322. Neither *Hin*dIII nor *Eco*RI cleaves the *gnd* gene of *E. coli* K-12 (26). Gna^+ transformants of strain GB23152 were selected on gluconate minimal agar medium. Colonies appearing after 5 to 14 days of incubation were purified on selective medium. Strains that contained gnd^+ plasmids were distinguished from those with edd^+ plasmids by screening whole-cell sonic extracts of ampicillin-resistant (Ap^r) clones for 6PGD activity with cellulose acetate electrophoresis. Figure 1 shows the mobility patterns for the isogenic *E. coli* K-12 strains and the respective plasmid transformants. The *gnd*(558) and *gnd*(B/r) genes were cloned as 6.5-kilobase (kb) *Eco*RI restriction fragments, and the *gnd*(567), *gnd*(740), and *gnd*(745) genes were cloned as *Hin*dIII restriction fragments of 3.5, 8.0, and 8.5 kb, respectively. The *gnd* allele of *S. typhimurium* LT-2 strain SB3436 was cloned as an 8.8-kb *Eco*RI restriction fragment, in agreement with the results of Bhaduri et al. (8).

Restriction mapping. The newly cloned *gnd* genes were mapped with 12 restriction endonucleases. The restriction maps are shown in Fig. 2. To locate and orient *gnd* on these DNA molecules, selected restriction fragments were subcloned into bacteriophage M13 RF DNA and the resulting recombinant M13 phages (Table 3) were then screened by a hybdridization test (5, 21) with single-stranded DNA carrying the sense or antisense strands of *gnd*(K-12) (25). The maps were aligned with respect to the conserved *Kpn*I and *Pst*I cleavage sites (Fig. 2), which span codons 124 and 370, respectively, in the *gnd*(K-12) gene (25). The aligned maps show that all the *gnd* genes differ from one another, except *gnd*(B/r) and *gnd*(558), which appear to be identical.

Restriction site polymorphisms were used to estimate the degree of difference between the respective *gnd* alleles (Table 5). The relative divergence was calculated by dividing the number of restriction site differences between each pair of alleles by six times the total number of sites present in the two genes. The rationale was as follows. (i) A single base pair change in a given sequence can result in either the gain or loss of a restriction site. (ii) Since each restriction enzyme had a 6-bp recognition sequence, the total number of base pairs examined was six times the number of restriction sites. Thus, for example, the relative divergence between the *gnd* genes of *E. coli* K-12 and *S. typhimurium* LT-2 was $4/(6 \times 6) = 0.111$.

DISCUSSION

In the present report, we have described an isogenic set of *E. coli* K-12 strains with a *gnd* allele from one of four *E. coli* natural populations or *E. coli* B/r strain NC3. Growth-rate-dependent regulation of *gnd* expression in the strains was assessed. The enzyme activity varied among the strains. Differences in enzyme activity of more than 50% are probably significant and most likely represent differences in the amount of 6PGD present in the cells, as shown previously in *E. coli* K-12 (37).

The level of 6PGD varied over a sixfold range in acetate-grown cultures of the isogenic strains (Table 4). Assuming that the different genes are regulated by the same mechanism as the *E. coli* K-12 allele, the differences in this basal level of expression should be due to differences in *gnd* mRNA level, presumably because of differences in promoter strength (2, 36). As discussed below, the differences could also be due to differences in flanking sequences that can act at a distance and are not *gnd* specific.

With respect to growth-rate-dependent regulation, the level of 6PGD in the isogenic strains was always higher in cells grown on glucose than in cells grown on acetate. Hence, the *gnd* alleles from natural populations and *E. coli* B/r are qualitatively similar to the previously characterized gene of *E. coli* K-12 (37) in their capacity to be regulated by growth rate. The magnitude of the growth rate induction of 6PGD level ranged from 1.8- to 3.4-fold. Assuming that the mechanism of growth rate control of the non-*E. coli* K-12

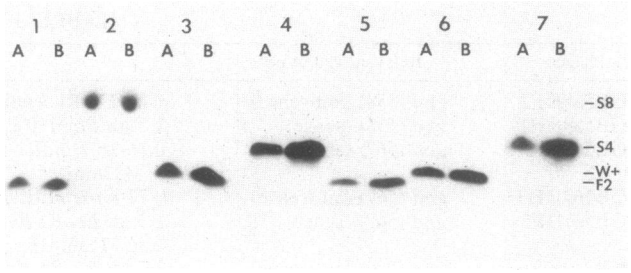

FIG. 1. Cellulose acetate electrophoretograms of native 6PGD enzyme activity. Electrophoresis of whole-cell sonic extracts prepared from LB broth cultures is described in the text. Lanes marked A show 6PGD activity in isogenic *E. coli* K-12 strains and in *S. typhimurium* LT-2. Lanes marked B show 6PGD activity from sonic extracts diluted 10- to 15-fold from the corresponding plasmid strains. Lanes: 1, GB310558 and GB23152(pGB310558); 2, GB310567 and GB23152(pGB310567); 3, GB310740 and GB23152 (pGB310740); 4, GB310745 and GB23152(pGB310745); 5, GB310B/r and GB23152(pGB310B/r); 6, W3110 and RW231(pMN1); 7, SB3436 and GB23152(pGB3436E). S8, S4, and F2 refer to electrophoretic mobility classes of 6PGD allozymes that are slower and faster, respectively, than the W^+ class, which has the same mobility as *E. coli* K-12.

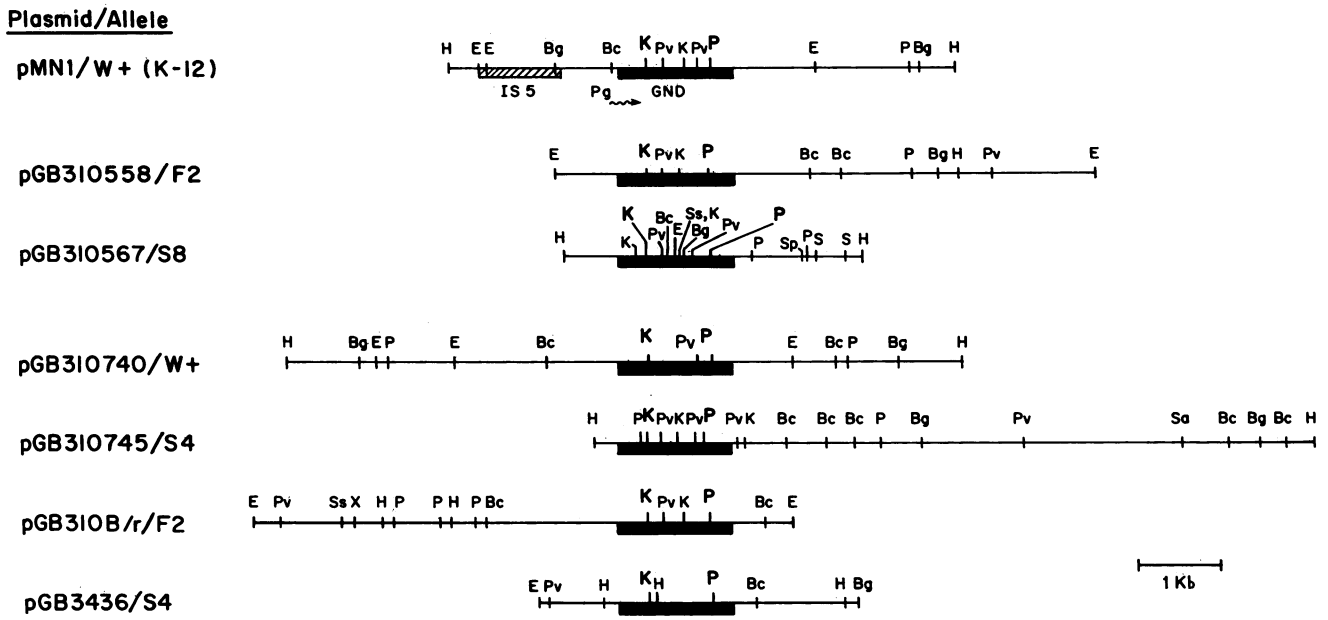

FIG. 2. Restriction endonuclease cleavage maps of plasmids carrying *gnd* alleles. All of the *gnd* genes were cloned into pBR322 as either *Eco*RI or *Hin*dIII restriction fragments. For mapping the *S. typhimurium gnd* gene, a 4.2-kb *Eco*RI-*Bgl*II fragment that carried all of the gene was subcloned from the initial plasmid into pBR322. Abbreviations: Bc, *Bcl*I; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dII; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*II; Sa, *Sal*I; S, *Sma*I; Sp, *Sph*I; Ss, *Sst*I; X, *Xba*I. **K** and **P** represent restriction endonuclease cleavage sites conserved among all alleles. Symbols: ▬, the 6PGD-coding region; Pg, promoter for *gnd*; ⇝, the direction of *gnd* transcription; ▨, IS*5* element. The maps are drawn to scale.

gnd alleles is similar to that of the *gnd*(K-12) gene (2, 3, 36), *gnd* mRNA produced from the alleles with the higher growth rate induction ratios might be more stable or translated more efficiently than mRNA from the alleles that are less growth rate inducible. In the accompanying paper (4), we present the nucleotide sequences of the regulatory regions of the various *gnd* genes and discuss the relationships between sequence features and *gnd* expression.

The conservation of growth rate inducibility of *E. coli gnd* alleles from widely diverse sources suggests that the growth-rate-dependent regulation is physiologically important. We imagine that the capacity for growth-rate-dependent regulation of this central metabolism gene is of selective advantage to the organism when it is faced with adjusting its growth rate to changes in the nutritional quality of its environment.

It is interesting to consider whether the differences in the amount of 6PGD activity produced by the different *gnd* alleles in *E. coli* K-12 would be physiologically important if they were produced in the same amounts in the corresponding naturally occurring strains. Although this was not done, two other studies have already examined similar *gnd* alleles. Miller et al. (24) selected a mutant in a gluconate-limited chemostat that had a faster growth rate than the input parental strain, which was a derivative of *E. coli* K-12 carrying the *gnd* gene from strain RM72B, i.e., the S4 allele. The chemostat-evolved strain proved to have threefold higher levels of 6PGD than the parent, and the mutation responsible for the elevation was a deletion just upstream from the *gnd* promoter. This would suggest that the differences we observed in enzyme level produced from the different *gnd* alleles might be of physiological significance to the organisms from which these alleles were derived. However, the starting strain in the directed evolution experiment carried an *edd* mutation, which forced metabolism of gluconate through the 6PGD reaction. Thus, since the growth properties of an *edd*$^+$ derivative of the evolved strain were not determined, these data cannot be directly applied to the question of the role of differences in 6PGD level in natural populations of *E. coli*.

On the other hand, Hartl and Dykhuizen (19) prepared a set of isogenic strains similar to the ones described here and examined the fitness of certain pairs in gluconate-limited

TABLE 5. Relative divergence of various *gnd* alleles[a]

gnd allele	Allele(s) derived from:						
	E. coli						*S. typhimurium*
	gnd(K-12)	*gnd*(558)	*gnd*(567)	*gnd*(740)	*gnd*(745)	*gnd*(B/r)	*gnd*(3436)
gnd(K-12)		0.033	0.083	0.067	0.028	0.033	0.111
gnd(558)			0.100	0.100	0.055	0.000	0.100
gnd(567)				0.116	0.091	0.100	0.136
gnd(740)					0.083	0.100	0.083
gnd(745)						0.055	0.119
gnd(B/r)							0.100

[a] Relative divergence was calculated as described in the text from the restriction maps in Fig. 3.

chemostats, conditions under which they argued the selective pressure on the alleles would be maximal. Among the particular combinations tested that involved the K-12, W^+, F2, and S4 alleles, there was no evidence for selection; the S8 allele showed a complex selectivity (19). These data, which were obtained with *edd*$^+$ strains, suggest that the differences in enzyme level that we observed with strains carrying these same alleles are not likely to be of direct physiological importance, although as argued by Dykhuizen and Hartl (13), they may have the potential for a selective advantage.

The restriction maps of the chromosomal DNA fragments differ for the various *gnd* alleles (Fig. 2). The size heterogeneity of the cloned restriction fragments and the extensive restriction site polymorphisms in the regions surrounding *gnd* suggest that the genetic recombination events that took place during the preparation of the isogenic *E. coli* K-12 strains involved DNA sequences that flank *gnd* at least several hundred base pairs both 5′ and 3′ to the structural gene. Thus, although the strains may not be truly isogenic for flanking regions, their respective *gnd* genes should be intact.

We previously noted that an IS*5* element is located about 0.5 kb upstream from the *gnd* promoter in *E. coli* K-12 (B. A. Jordan, R. E. Wolf, Jr., M. S. Nasoff, Plasmid **8**:98, 1982). No other cloned *gnd*$^+$ chromosomal fragment (Fig. 2) has *Eco*RI and *Bgl*II restriction cleavage sites characteristic of IS*5* (15, 31). Thus, among these *E. coli* strains, only *E. coli* K-12 has an IS*5* element near *gnd*. Moreover, since IS*5* is able to activate gene expression from a distance of more than 100 base pairs (29, 30), it is possible that the element is responsible for some of the difference in expression between *gnd*(K-12) and the other *gnd* alleles.

The absence of an IS*5* element near *gnd* in strain GB310745 conflicts with data of Miller et al. (24). They also used an *E. coli* K-12 strain in which the K-12 gene had been replaced by the gene from strain RM72B, which should be analogous to our strain GB310745. Upon cloning and restriction mapping of the *gnd* gene from the evolved strain and from its parent, Miller et al. (24) found that an IS*5* element was present upstream from the *gnd* promoter in both strains; the distance between the IS*5* element and the *gnd* promoter in their S4-allele-containing parental strain is the same as in *E. coli* K-12 (24; unpublished data). A comparison of the restriction maps suggests that the strain used by Miller et al. (24) actually contained a chimeric *gnd* gene which was composed of the coding sequence of the gene from strain RM72B and the promoter and 5′- and 3′-flanking DNA of the *E. coli* K-12 gene; presumably this hybrid gene arose during one of the transductions used in its construction. The restriction map of the *gnd* gene cloned directly from strain RM72B (unpublished results) supports this conclusion.

The coding sequences of the various *gnd* alleles have unique restriction maps (Fig. 2). In fact, only the *Kpn*I and *Pst*I sites at codons 124 and 370, respectively, were conserved. The degree of divergence was estimated from the restriction site polymorphisms (Table 5). On the basis of this analysis, the *gnd* alleles of *E. coli* may be grouped as follows. The *gnd*(558) and *gnd*(B/r) alleles appear to be identical. The *gnd*(K-12) and *gnd*(745) alleles differ from each other and from the above two alleles by about 3%. The *gnd*(740) allele differs from the previous four alleles by about 9%, whereas the *gnd*(567) allele is the most distant from the other *E. coli* alleles, differing about 10% from each allele. The *S. typhimurium gnd* gene differs from the *E. coli* genes by about 11%. These data are in good agreement with results obtained by Harshman and Riley (18) in their analysis of restriction fragment length polymorphisms in the *trp* operon of natural isolates of *E. coli*. For 26 of 28 strains investigated, the frequency of nucleotide substitution averaged 3%, with a range of 0.8 to 6.6% (18). In the accompanying paper (4), we analyze the nucleotide sequences of the *gnd* alleles described here.

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