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Divergent Transcription of the *sn*-Glycerol-3-Phosphate Active Transport (*glpT*) and Anaerobic *sn*-Glycerol-3-Phosphate Dehydrogenase (*glpA glpC glpB*) Genes of *Escherichia coli* K-12

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The *glpTQ* operon and the *glpA* and *glpB* genes are located adjacent to one another near min 49 of the linkage map of *Escherichia coli* K-12. The positions and directions of transcription of the *glpA* and *glpB* genes with respect to the *glpTQ* operon were determined in the present work. Strains harboring Mu d1(Ap *lac*) fusions in either *glpA* or *glpB* were converted to the respective λ *p*1(209) lysogens. Induction of these lysogens with mitomycin C resulted in production of Lac$^+$ phage progeny which carried adjacent chromosomal DNA. Genetic crosses with a collection of *glpT* mutant strains were performed with several such phage lines. A fine-structure deletion map of the *glpT* gene was thus constructed. All phages used for this mapping carried DNA starting with the promoter-proximal end of *glpT*. This indicated that the *glpTQ* operon and the *glpA* and *glpB* genes are transcribed divergently. Additional evidence supporting this conclusion was obtained by physical mapping of restriction endonuclease cleavage sites in plasmids carrying these genes and in plasmids carrying *glpA-lacZ* or *glpB-lacZ* fusions. A new designation (*glpC*) for the gene encoding the 41,000-M_r subunit of the anaerobic *sn*-glycerol-3-phosphate dehydrogenase was proposed to distinguish it from the *glpA* gene, which encodes the 62,000-M_r subunit of the dehydrogenase, and the *glpB* gene, which encodes a membrane anchor subunit of the dehydrogenase. These three genes were present in an operon transcribed in the order *glpA glpC glpB* in the clockwise direction on the linkage map of *E. coli*.

The *glp* regulon of *Escherichia coli* encodes the proteins required for the dissimilation of glycerol, glycerol-3-phosphate (glycerol-P), and glycerophosphodiesters (13, 17). The operons which comprise this regulon are found at three locations on the linkage map of *E. coli* (1). The *glpK* and *glpF* genes, encoding glycerol kinase and glycerol diffusion facilitator, respectively, map near min 88. The *glpD* and *glpR* genes, encoding aerobic glycerol-P dehydrogenase and the *glp* repressor, map near min 75. Two operons are found near min 49. The *glpTQ* operon encodes the cytoplasmic membrane-associated glycerol-P permease (15) and the periplasmic glycerophosphodiester phosphodiesterase (13). The *glpA* and *glpB* genes map very close to *glpTQ* and encode components of the anaerobic glycerol-P dehydrogenase (12). The *glpA* gene encodes a catalytic component of the enzyme; *glpB* is defined as a gene encoding a membrane anchor subunit for the enzyme (12). It is not clear whether the two genes are organized in a single transcription unit. If they are, *glpA* is likely to be promoter proximal to *glpB*, because insertions in *glpB* do not inactivate *glpA* enzyme function measured in vitro. It is not clear where the *glpAB* genes are located with respect to the *glpTQ* operon, although evidence has been obtained indicating that they are not found in the same transcription unit with *glpT* (20).

In a previous study, we found that *glpT* is transcribed in a counterclockwise direction on the chromosome. Furthermore, deletion analysis placed the *glpA* locus downstream from *glpT* (18). The results of a later study cast some doubt on such placement of *glpA*, however. It was found that DNA located upstream with respect to the *glpT* promoter encoded a 62-kilodalton protein (15). This is the same size as one of the subunits present in a purified preparation of the anaerobic glycerol-P dehydrogenase (22; the preparation also contained a 43-kilodalton protein). Proteins of the same size were subsequently identified as the *glpA* gene products by using recombinant plasmids carrying DNA from this region of the chromosome (23).

The results reported in this paper reconcile these contradictory findings. The results clearly show that the *glpA* and *glpB* genes are located directly adjacent to and are transcribed divergently from the *glpTQ* promoter. In addition, the genes encoding the 62-kilodalton, 43-kilodalton, and membrane anchor subunits of the dehydrogenase (named *glpA*, *glpC*, and *glpB*, respectively) were found to constitute an operon transcribed in the order *glpA glpC glpB*.

MATERIALS AND METHODS

Bacterial strains, phages, and growth media. Strains harboring the mutant *glpT* alleles mapped during the course of this work have been described (15, 18). The other strains utilized or constructed are listed in Table 1. Preparation of phage lysates and transductions were carried out as described by Silhavy et al. (25).

For minimal media, the A and B salts of Clark and Maaløe (6) were supplemented with 0.2 to 0.4% of the various carbon sources and 2 μg of thiamine per ml. Testing of *glpACB* phenotypes was done anaerobically with minimal medium containing 0.4% glycerol, 20 mM sodium fumarate,

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TABLE 1. Strains of *E. coli* K-12

Strain	Genotype	Source
NM303	*lacZ mgl recA1* (λ)	N. Müller
MAL103	F⁻ Δ (*gpt-proAB-argF-lac*)XIII *rpsL* [Mu dl(Ap *lac*)] (Mu cts62)	4
ECL8	HfrC *phoA8 glpD3 glpR2 relA1 spoT1 fhuA22 ompF627 fadL701 pit-10* (λ)	7
MC4100	F⁻ *araD139* Δ(*argF-lac*)*U169 rpsL150 deoC1 relA1 rbsR ptsF25 flbB5301*	3
TST3	MC4100 *malT*::Tn*10*	25
DL13	MC4100 *glpR2 gyrA zei-725*::Tn*10*	18
ECL381	MC4100 *sdh-9* Δ*glpD102 glpA101*::Mu dl	12
ECL384	MC4100 *sdh-9* Δ*glpD102 glpA104*::Mu dl	12
ECL392	ECL381 Δ(Mu dl) [λ *p*1(209)]	9
TL45	MC4100 *glpR2* Δ(*glpT-glpA*)*593 gyrA*	18
TL48	MC4100 *glpD3 glpR2*	14
TL52	TL45 *glpD3 glpR2*	This work
TL58	MC4100 *glpT658*::Mu dl	This work
TL59	MC4100 *glpB659*::Mu dl	This work
TL60	MC4100 *glpB660*::Mu dl	This work
TL61	MC4100 *glpB661*::Mu dl	This work
TL658	TL58 Δ(Mu dl) [λ *p*1(209)]	This work
TL659	TL59 Δ(Mu dl) [λ *p*1(209)]	This work
TL660	TL60 Δ(Mu dl) [λ *p*1(209)]	This work
TL661	TL61 Δ(Mu dl) [λ *p*1(209)]	This work
TL667	TL67 Δ(Mu dl) [λ *p*1(209)]	18
TL668	TL68 Δ(Mu dl) [λ *p*1(209)]	18
TL669	TL69 Δ(Mu dl) [λ *p*1(209)]	18
TL681	ECL381 Δ(Mu dl) [λ *p*1(209)]	This work
TL684	ECL384 Δ(Mu dl) [λ *p*1(209)]	This work
ME8	TL658 *glpD3 glpR2*	This work
ME9	TL659 *glpD3 glpR2*	This work
ME10	TL660 *glpD3 glpR2*	This work
ME11	TL661 *glpD3 glpR2*	This work

and 0.03% Casamino Acids (Difco Laboratories). Anaerobic conditions were obtained by using a sealed jar with an anaerobic gas pack (BBL Microbiology Systems). LB medium (21) was used for routine liquid cultures, and TB medium (25) was used for work with λ. Nutrient agar (Difco) containing 20 mM sodium citrate was used for selection of Nal^r or Tc^r transductants in phage P1-mediated crosses. The lactose phenotype of strains carrying *glp-lac* fusions was determined with MacConkey-lactose medium (21) or on various minimal media containing 40 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside per ml. When needed, antibiotics were added at the following concentrations: tetracycline, 5μg/ml; ampicillin, 25 or 50 μg/ml; and nalidixic acid, 50 μg/ml.

The *glpD3 glpR2* alleles of strain ECL8 were transfered into other strains by using two P1 transductions (14). Strain TST3 (*malT*::Tn*10*) served as the first P1 donor (selection for Tc^r), and strain ECL8 (*glpD3 glpR2*) served as the second P1 donor (selection for Mal$^+$).

Isolation of *glp-lac* fusion strains. Fusions of *glp* promoters to the lactose genes was accomplished by infection of strain MC4100 with Mu dl(Ap *lac*) phage prepared by thermal induction of strain MAL103 (4). Ap^r transductants were screened for those exhibiting glycerol-inducible lactose utilization by replica plating onto MacConkey-lactose-ampicillin medium with or without 0.1% glycerol (18). Strains harboring putative *glp-lac* fusions were purified and then tested on minimal medium containing either glycerol, glycerol-P, glycerophosphocholine, or glycerol plus furmarate (anaerobically).

Isolation of specialized transducing phages carrying *glp-lac* fusions. The Mu dl(Ap *lac*) insertions in the above strains and in strains ECL381, ECL382, and ECL384 (all *glpA-lac* fusions) were replaced by λ *p*1(209) as described previously (11, 25). Several phage lines carrying the *glp-lac* fusions were isolated after mitomycin C induction of the λ *p*1(209) lysogens TL658-661 and TL681-684. Some of the phage progeny formed blue plaques on lawns of TL45 spread on TB–5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside plates. These plaques were suspended in 0.1 ml of λ dilution buffer. To find out whether these phages carried *glpT* material, crosses with three *glpT* mutant strains were performed. Strains harboring *glpT502*, *glpT512*, and *glpT520* were grown overnight in TB, harvested, and suspended in 10 mM $MgSO_4$ (one half the culture volume). A 0.1-ml sample of this cell suspension mixed with 2.5 ml of F top agar (21) was plated on minimal glycerol-P medium. Ten microliters of the above phage suspensions was then spotted on these lawns, and incubation at 37°C was carried out for 2 days. Phages giving positive results with one or more of the *glpT* mutants were purified several times on TB–5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside plates with TL45 as the host. High-titer lysates were then prepared, and the crosses were repeated with the complete set of *glpT* mutant strains (15, 18).

Mapping of *glpT* mutations with *glp-lac* transducing phages. Crosses between *glpT* mutant strains and transducing phages derived from *glp-lac* fusion strains were performed by cross-streaking the *glpT* mutant strains against 0.025 ml of high-titer phage lysates on solidified glycerol-P minimal medium (two lysates per plate). Results were recorded after 2 days of incubation at 37°C. Results reproduced in three separate experiments are indicated in Fig. 1.

Preparation of crude extracts. Cells were grown aerobically overnight in 100 ml of the indicated medium and harvested by centrifugation. After washing with 25 ml of 50 mM potassium phosphate (pH 7.5), 1 mM $MgCl_2$, and 1 mM 2-mercaptoethanol, cells were suspended in 10 ml of the same buffer and passed through a French press. The crude extract is the supernatant fraction resulting from centrifugation at 10,000 × *g* for 20 min at 4°C.

Protein concentrations were estimated by the method of Bradford (2) with bovine serum albumin as the standard.

Enzyme assays. Anaerobic glycerol-P dehydrogenase activity was measured at 30°C with the continuous spectrophotometric assay of Kistler and Lin (10). Activity was dependent upon added flavins and glycerol-P and was linear with respect to time and protein concentration.

β-Galactosidase activity was determined as described by Miller (21).

DNA manipulations. Phage λ DNA was prepared from 100-ml lysates by the procedure of Silhavy et al. (25). Plasmid DNA was isolated by a sodium dodecyl sulfate alkaline lysis procedure (25). Restriction endonuclease cleavage analysis and ligation reactions were carried out as previously described (24).

RESULTS

Isolation and characterization of *glp-lac* operon fusions. Fusion of the *lac* structural genes to *glp* promoters was achieved by testing approximately 3,000 Mu dl(Ap *lac*) transductants for glycerol inducibility of lactose utilization using MacConkey-lactose indicator medium as described in Materials and Methods. Lysogens exhibiting the desired phenotype were purified and then tested on appropriate minimal media to determine whether any of the isolates

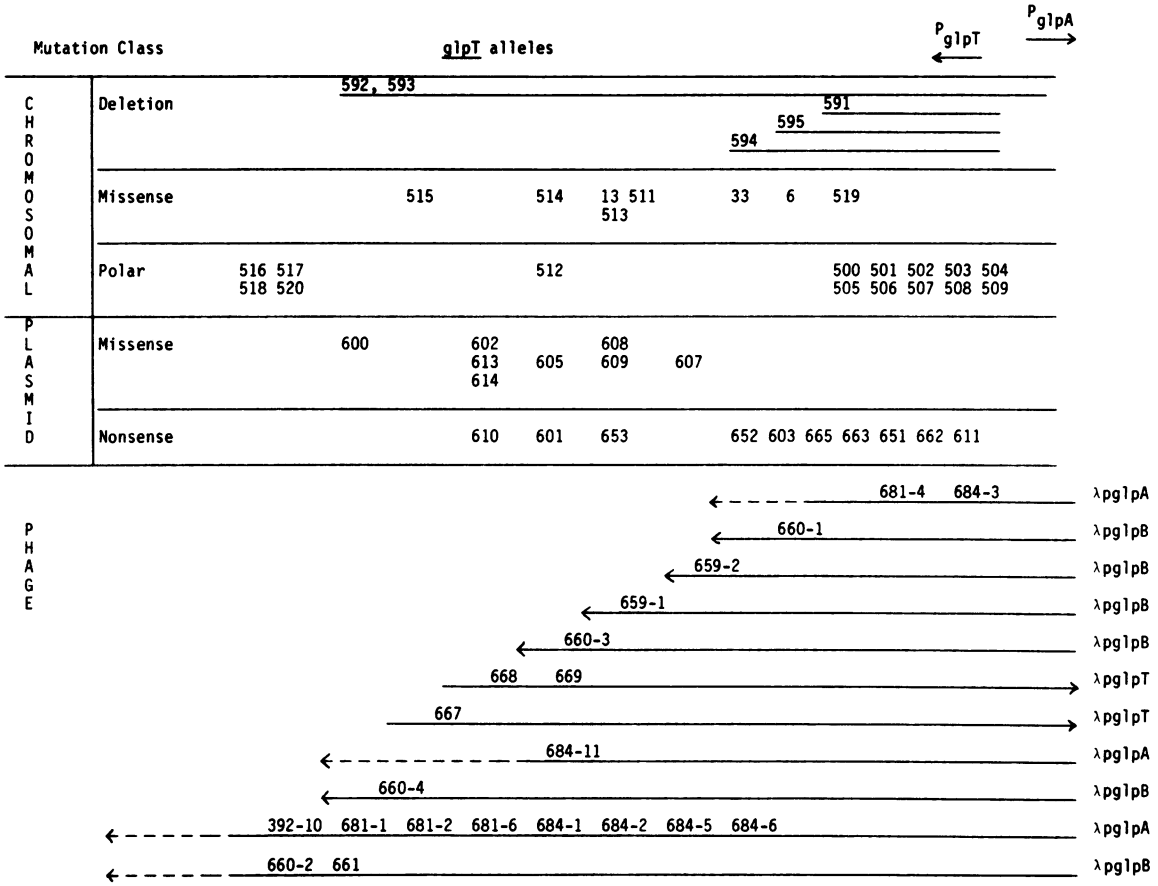

FIG. 1. Genetic map of *glpT*. Mutant strains harboring the indicated chromosomal or plasmid-borne *glpT* alleles were crossed with phages derived from strains with λ integrated at *glpA-lac*, *glpB-lac*, or *glpT-lac* fusions as indicated on the right. Phages carried chromosomal DNA shown as a solid line. The dashed extensions indicate uncertainty about endpoints. Arrows point away from *glp-lac* fusion joints. The first three numbers for a phage isolate indicate the bacterial strain from which it was derived; numbers after the hyphens are isolate numbers. The chromosomal and plasmid-borne mutations mapped were those described earlier (15, 18). The chromosomal deletions are indicated by the solid lines at the top of the drawing. Point mutations were divided into two classes; they were considered missense mutations if they had no polar effect on expression of *glpQ* and were not suppressed by amber or ochre suppressors. All of the nonsense mutations carried by the plasmid pGS31 are amber mutations except for *glpT601* and *glpT611*.

carried fusions to known *glp* genes. One colony (TL58) was unable to grow on glycerol-P or glycerophosphocholine and thus contained an insertion in *glpT*. Three isolates (TL59, TL60, and TL61) were unable to grow anaerobically on glycerol plus fumarate and hence appeared to contain insertions in a gene encoding a component of the anaerobic glycerol-P dehydrogenase.

The positions of the Mu dl(Ap *lac*) insertions in these strains were determined by P1 transduction with DL13 (*zei-725*::Tn*10*) as the donor (selection for Tc^r). In each case, 60 to 80% of the Tc^r transductants became Ap^s Lac^-. This is the expected cotransduction frequency between this Tn*10* and *glpT* (18). Therefore, all three fusions are located in the *glpA-glpT* region of the chromosome.

To find out whether anaerobic glycerol-P dehydrogenase activity is impaired in these *glp-lac* fusion strains, the activity was measured in crude extracts. First, however, the *glpD3 glpR2* alleles were introduced into strains TL659, TL660, and TL651 by P1 transduction, yielding strains ME9, ME10, and ME11, respectively. The *glpD3* allele prevents interference by aerobic glycerol-P dehydrogenase during assay of the anaerobic dehydrogenase, and *glpR2* allows constitutive expression of the *glp* regulon, including anaerobic glycerol-P dehydrogenase. Crude extracts were prepared from the above strains and control strains. Data presented in Table 2 show that anaerobic glycerol-P dehydrogenase levels in strains ME9, ME10, and ME11 were comparable to the levels found in the $glpA^+$ control strains TL48 and ME8. Thus, the *glp-lac* fusions in strains TL59, TL60, and TL61 (the parent strains) resemble the *glpB-lac* fusions described by Kuritzkes et al. (12). Insertions in *glpB* impair anaerobic growth on glycerol plus fumarate but do not abolish enzyme activity measured in vitro. The *glpB* gene is thought to encode a membrane anchor subunit of the enzyme, because *glpB* mutant strains accumulate active enzyme in the cytoplasmic fraction (12). The enzyme in wild-type strains is loosely associated with the membrane (10, 22).

Mapping of *glpA-lac* and *glpB-lac* fusions with respect to *glpT*. The availability of *lac* fusions in a given gene provides the opportunity to carry out fine structure genetic analysis of nearby genes, as used by Silhavy et al. (26) for the divergently transcribed operons of the *malB* region. To perform such analysis, the Mu dl(Ap *lac*) lysogens are first converted to λ lysogens by using λ *p*1(209), which integrates at the fusion via Mu or *lac* homology (25). Subsequent mitomycin

TABLE 2. Anaerobic glycerol-P dehydrogenase activity in *glp-lac* fusion strains

Strain[a]	Relevant genotype[b]	Sp act[c]
TL48	Wild type	52
TL52	Δ(*glpT-glpA*)*593*	ND
ME8	*glpT658*::Mu dl	28
ME9	*glpB659*::Mu dl	18
ME10	*glpB660*::Mu dl	22
ME11	*glpB661*::Mu dl	24

[a] The strains were grown overnight on LB medium.
[b] All strains contained the *glpD3 glpR2* alleles.
[c] Enzyme specific activity is expressed as nanomoles of glycerol-P oxidized per minute per milligram. ND, No activity above background was detected.

C induction of λ results in phage progeny which may carry adjacent chromosomal material. Deletion mapping is possible because different phages excise different amounts of adjacent chromosomal DNA.

Because *glpA* and *glpT* are very tightly linked (1, 17), and because a series of strains carrying defined *glpT* mutations is available (18), we reasoned that *glpA* and *glpB* fusions might be used in experiments analogous to those used for mapping of the divergent *malEFG* and *malK lamB* operons (26), especially if divergent operons are involved. Thus, strains TL59, TL60, and TL61 (all *glpB-lac*) and strains ECL381 and ECL384 (both *glpA-lac*) were converted to λ lysogens and named TL659, TL660, TL661, TL681, and TL684 respectively. Mitomycin C induction of these lysogens resulted in production of Lac$^+$ phage progeny. These were tested to see whether they carried any *glpT* DNA. Indeed, some of the Lac$^+$ phages from both *glpA-lac* and *glpB-lac* fusions were found to carry portions of *glpT* (Fig. 1). In all cases, phages were isolated which carried *glpT* material starting at the promoter-proximal end of *glpT* (18). At least two different phages were isolated from each of the *glpA* or *glpB* fusion strains. For example, strain TL684 yielded phage lines 684-3, 684-11, 684-1, each carrying increasing amounts of *glpT* DNA starting from the promoter-proximal end of *glpT*. These results demonstrate that *glpA* and *glpB* are located to the right of the *glpT* promoter as shown in Fig. 1. In addition, the results suggest that both *glpA* and *glpB* are transcribed divergently from the *glpT-glpQ* operon. Thus, the *glpA* and *glpB* genes are transcribed in the clockwise direction on the chromosome, because *glpT* is transcribed in the counterclockwise direction (18).

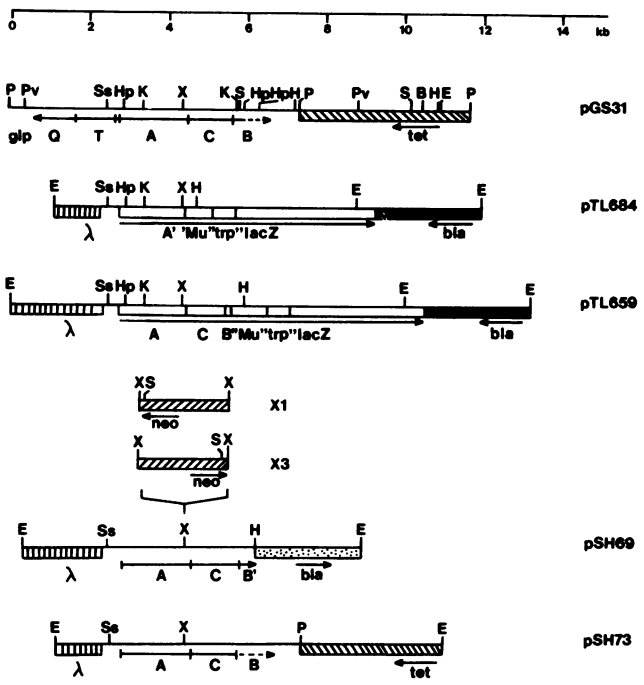

FIG. 2. Restriction maps of plasmids carrying portions of the *glpT-glpA* region of the *E. coli* chromosome. The restriction endonucleases used were *Bam*HI (B), *Eco*RI (E), *Hin*dIII (H), *Hpa*I (Hp), *Kpn*I (K), *Pst*I (P), *Pvu*II (Pv), *Sal*I (S), *Sst*II (Ss), and *Xho*I (X). The *bla* gene of pMLB524 (■) and pUC18 (▩) encodes β-lactamase; *tet* indicates the tetracycline resistance gene of pBR322 (▨); *neo* encodes neomycinphosphotransferase II contained on a 2.4-kilobase(kb) *Xho*I fragment (▨) derived from Tn*5* (19). The approximate amount of λ DNA (▥) carried by the different plasmids is also indicated.

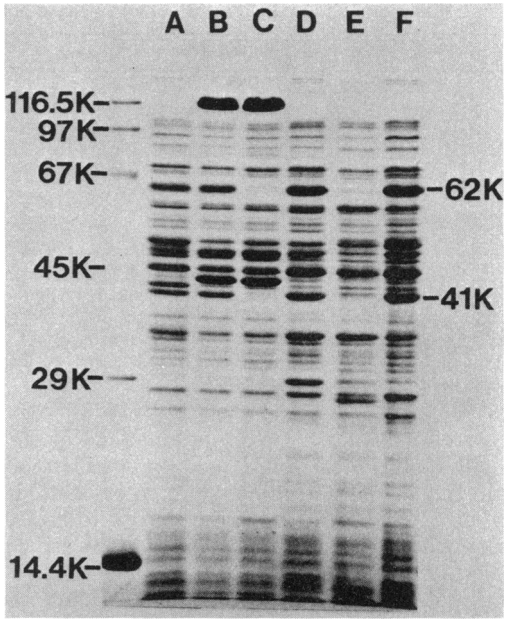

FIG. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of crude extracts from TL52 harboring various plasmids. Sample preparation and electrophoresis on a 12% polyacrylamide gel were carried out as described previously (15). TL52 contained the following plasmids: A, pGS31; B, pTL659; C, pTL684; D, pSH69; E, pSH69-X3; F, pSH73. The molecular masses (kilodaltons) of protein standards (β-galactosidase, phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, and lysozyme) are indicated on the left. The positions of the *glpA* and *glpC* gene products are indicated on the right. The gel was stained with Coomassie blue.

In addition to providing information about the directions of transcription of the *glpT*, *glpA*, and *glpB* genes, the phages described above were useful for mapping of plasmid-borne mutations generated by hydroxylamine mutagenesis in vitro (15). For these experiments, the plasmids were introduced into TL45 [Δ (*glpT-glpA*) *593*]. The resulting transformants were cross-streaked against the indicated phage lysates (Fig. 1). The order of the amber mutations correlated well with the sizes of the amber fragments synthesized in vitro (15), providing additional evidence in support of the proposed direction of transcription of *glpT*. Mapping of these amber mutations to a given deletion interval allowed prediction of the physical locations of other mutations. For example, most of the plasmid-borne missense mutations

TABLE 3. Plasmid-encoded glycerol-P dehydrogenase activity

Plasmid[a]	Plasmid genotype	Sp act[b]
pGS31	*glpA*$^+$*C*$^+$ (*B*$^+$)[c]	3,590
pTL659	*glpA*$^+$*C*$^+$ (*glpB-lacZ*)	2,860
pTL684	*glpA-lacZ*	ND
pSH69	*glpA*$^+$*C*$^+$	3,800
pSH69-X1	*glpA*::*neo glpC*$^+$	ND
pSH69-X3	*glpA*::*neo glpC*$^+$	ND
pSH73	*glpA*$^+$*C*$^+$ (*B*$^+$)	3,420

[a] Strain TL52 [Δ(*glpT-glpA*)*593 glpD3 glpR2*] harboring the indicated plasmids was grown overnight on LB medium supplemented with the appropriate antibiotic.

[b] Enzyme specific activity and ND are defined in Table 2.

[c] Parentheses indicate the presence of the *glpB* gene has not been confirmed, but is implied by the physical maps.

were clustered near the carboxyl-terminal end of *glpT* (see Fig. 3). All of these missense mutations except *glpT607* mapped in the same or in a more promoter-distal deletion interval than *glpT653*(Am), which gives rise to a 27,000-M_r amber fragment (15). The G3P permease has an apparent molecular weight of 33,000 (15).

Cloning and physical characterization of DNA carrying *glpA-lac* and *glpB-lac* fusions. To conclusively demonstrate that *glpA* and *glpB* are transcribed divergently from *glpT*, DNA carrying fusions to these genes was subjected to restriction endonuclease cleavage analysis. DNA was isolated from λ 684-11 (*glpA-lac*) and from λ 659-2 (*glpB-lac*) (Fig. 1). *Eco*RI fragments harboring the fusions were subcloned into pMLB524, a vector for cloning of *lacZ* fusions (25), resulting in pTL684 and pTL659, respectively (Fig. 2). Comparison of the restriction maps of these two plasmids with that for pGS31 (where the direction of transcription of *glpT* is known [15]) clearly demonstrates that both *glpA* and *glpB* are transcribed divergently from *glpT* (Fig. 2). The *Hpa*I, *Kpn*I, and *Xho*I sites were present in all of these plasmids.

Crude extracts of strain TL52 [Δ (*glpT-glpA*) *593 glpD3 glpR2*] harboring the above plasmids were prepared, and anaerobic glycerol-P dehydrogenase specific activity was determined. pTL659 encodes high levels of glycerol-P dehydrogenase (Table 3), as might be predicted from the results obtained from strain ME9, which harbors the same *glpB-lac* fusion on the chromosome (Table 2). As expected, pTL684 (harboring a *glpA-lac* fusion) did not express glycerol-P dehydrogenase activity.

Organization of the genes encoding anaerobic glycerol-P dehydrogenase. Anaerobic glycerol-P dehydrogenase has been identified and isolated by Schryvers and Weiner (22, 23). The purified preparation contained two polypeptides with apparent molecular weights of 62,000 and 43,000. To determine the gene-protein relationships for the anaerobic glycerol-P dehydrogenase, the effect of insertions in *glpA* and *glpB* upon synthesis of these two proteins was assessed. These two proteins were identified by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of crude extracts of strain TL52 [Δ(*glpT-glpA*) *593*] harboring the appropriate plasmids. A crude extract of TL52(pGS31) (which carries the wild-type *glpTQ-glpACB* region [15]) contained large amounts of enzyme activity (Table 3) and large amounts of a 62,000-M_r and a 41,000-M_r protein (Fig. 3, lane A). A plasmid harboring an insertion in *glpB* (pTL659) also overexpressed glycerol-P dehydrogenase activity (Table 3) and directed the synthesis of the 62,000-M_r and the 41,000-M_r proteins (Fig. 3, lane B). An insertion in the *glpA* gene (pTL684) abolished enzyme activity and the synthesis of both proteins (Table 3; Fig. 3, lane C). The results suggest that *glpB* does not encode either of these two proteins. We therefore propose the genetic designations *glpA* and *glpC* for the 62,000- and 41,000-M_r proteins, respectively.

Additional plasmids were constructed so that the order of the genes encoding these two proteins could be determined. First, the *Eco*RI-*Hin*dIII fragment encoding glycerol-P dehydrogenase from pTL659 was cloned into pUC18 (28). The resulting plasmid was named pSH69 (Fig. 2). This plasmid encoded high levels of glycerol-P dehydrogenase activity (Table 3) and both the 62,000- and 41,000-M_r proteins (Fig. 3, lane D). To determine the positions of the genes encoding these two proteins and to determine whether the genes comprise an operon, the effect of insertion of an *Xho*I fragment into the *Xho*I site of pSH69 was assessed. Insertion of the 2.4-kilobase fragment derived from Tn*5* (19) into pSH69, in either orientation (Fig. 2), abolished synthesis of active enzyme (Table 3). Synthesis of the 62,000- and the 41,000-M_r proteins was also affected in the case of insertion X3 (Fig. 3, lane E). These results indicate that the *glpA* and *glpC* genes are present in the same operon.

The results also suggest that the gene encoding the 62,000-M_r subunit (*glpA*) is promoter proximal in the operon. This is so because the amount of DNA present between the *glpA* promoter and the *Xho*I site is more than enough to encode the 41,000-M_r subunit. If the gene encoding the 41,000-M_r component were promoter proximal, its synthesis should have been unaffected by the insertions at the *Xho*I site. The *glpA* promoter was localized to the left of the *Kpn*I and *Hpa*I sites (Fig. 2) by cloning of the appropriate DNA restriction fragments (data not shown). Placement of the gene encoding the 62,000-M_r protein in the promoter-proximal position is also supported by the results of Schryvers and Weiner (23). They isolated a plasmid which encodes a truncated *glpA* polypeptide of 58,000 M_r. The plasmid did not encode the 41,000-M_r *glpC* gene product. Also, a gene in *Salmonella typhimurium* located directly adjacent to the *glpTQ* promoter encodes a 62,000-M_r protein (8).

The *glpB* gene product, the membrane anchor (12), was not identified in these experiments. To rule out the possibility that the 41,000-M_r protein is a truncated protein resulting from insertion of Mu d1 near the carboxyl terminus of the gene encoding this protein, the proteins encoded by a plasmid (pSH73, Fig. 2) harboring the wild-type *glpACB* region were identified. pSH73 was constructed by replacing the *Eco*RI-*Xho*I fragment (harboring the *glpTQ* operon) of pGS31 with the *Eco*RI-*Xho*I fragment of pTL684 such that a functional *glpACB* operon would be reconstructed on a plasmid deleted for *glpTQ*. This plasmid expressed large amounts of enzyme activity (Table 3), and the sizes of the *glpA* and *glpC* gene products identified after sodium dodecyl sulfate-polyacrylamide electrophoresis (Fig. 3, lane F) were identical to those encoded by all of the other plasmids carrying *glpA*$^+$*C*$^+$. Thus, it is likely that the 62,000- and 41,000-M_r proteins are those encoded by the wild-type *glpA* and *glpC* genes, respectively, and that another gene (*glpB*) or genes encode the membrane anchor for the anaerobic glycerol-P dehydrogenase.

To determine whether *glpB* is present in the same operon with *glpA* and *glpC*, the 2.4-kilobase *Xho*I fragment from Tn*5* harboring the *neo* gene (19) was inserted into the *Xho*I site in the *glpA* gene of pTL659 (Fig. 2). The effect of insertion of this fragment in both orientations on the expression of the *glpB-lac* fusion was assessed by measuring β-galactosidase activity in strain NM303 (Δ*lacZ*) harboring pTL659, pTL659-X1, and pTL659-X3 (Table 4). The inser-

TABLE 4. Effect of insertions in *glpA* on *glpB-lacZ*-encoded β-galactosidase activity

Strain[a]	β-Galactosidase sp act[b] with the following addition to growth medium:		
	None	Glycerol	Glucose
NM303(pTL659)	1,500	9,260	160
NM303(pTL659-X1)	230	170	160
NM303(pTL659-X3)	2,000	1,100	1,220

[a] Strain NM303 (Δ*lacZ*) harboring the indicated plasmids was grown in M9 minimal medium (21) supplemented with 1% Casamino Acids and 100 μg of ampicillin per ml. Glycerol or glucose was added at 0.4% as indicated.

[b] β-Galactosidase specific activity is expressed as nanomoles of *o*-nitrophenolate produced per minute per milligram.

tion carried by pTL659-X1 caused a 50-fold reduction of β-galactosidase activity relative to that expressed from pTL659 under inducing conditions. Insertion of the fragment in the opposite orientation such that the *neo* gene is transcribed in the same direction as *glpB-lac* (pTL659-X3) allowed a lower-level, constitutive expression of β-galactosidase activity, probably due to readthrough from the *neo* promoter. The results indicate that *glpB* is present in the same transcription unit with *glpA*, because insertions in *glpA* have a strong polar effect on expression of *glpB*.

DISCUSSION

The results presented in this paper clearly demonstrate that the genes encoding anaerobic glycerol-P dehydrogenase are transcribed divergently from the *glpTQ* operon. Our previous placement of *glpA* downstream from *glpTQ* was based on the observation that strains harboring *glpT* deletions 591, 594, and 595 (Fig. 1) were *glpA*$^+$, and strains harboring *glpT* deletions 592 and 593 were *glpA* (18). We thought that these deletions removed all of the DNA between a Tn*10* located upstream with respect to the *glpTQ* promoter and an endpoint in *glpT* (18). Thus, it appeared that *glpA* could not be located between this Tn*10* and *glpT*. Deletions 592 and 593 were thought to impair *glpA* function by extending through the entire *glpT* gene into *glpA*. These deletions appeared to cover all of *glpT* because they were unable to recombine with any *glpT* point mutations in phage P1-mediated crosses (18).

The present mapping studies employing specialized λ *pglpA* and λ *pglpB* transducing phages proved that the above interpretation was incorrect. It is clear that both *glpA* and *glpB* are transcribed divergently from *glpTQ* because these phages carried different amounts of DNA at the promoter-proximal end of *glpT*. The results of Yamada et al. (27) support placement of *glpA* upstream with respect to the *glpTQ* promoter. They have physically mapped the *ftsB* and *nrdAB* genes directly downstream from *glpTQ*, precluding placement of *glpA* in this position.

It is apparent that the chromosomal deletions affecting *glpT* are not as extensive as previously believed (18). Point mutations *glpT516*, *glpT517*, *glpT518*, and *glpT520* have now been mapped to a more promoter-distal interval than the endpoints of the extensive deletions *glpT592* and *glpT593*. The deletions which did not affect *glpA* (*glpT591*, *glpT594*, and *glpT595*) could have resulted from DNA rearrangements such as inversion or inversion accompanied by deletion so that both *glpT* and the Tn*10* were affected.

Physical characterization of DNA carrying the *glpA-glpTQ* region of the chromosome provided conclusive evidence supporting the conclusion that the genes encoding the anaerobic glycerol-P dehydrogenase are divergently transcribed from the adjacent *glpTQ* operon. In addition, evidence was obtained indicating that a minimum of three genes is needed for anaerobic glycerol-P dehydrogenase function. The *glpA* and *glpC* genes encode the 62- and 41-kilodalton subunits of the enzyme, respectively. The *glpB* gene encodes a membrane anchor subunit of the enzyme (12). The *glpACB* genes comprise an operon with *glpA* promoter proximal. Thus, the *glpACB* operon is functionally analogous to the *frdABCD* operon, which encodes fumarate reductase (5, 16). The promoter-proximal *frdAB* genes encode the catalytic portion of the enzyme, and the distal *frdCD* genes encode integral membrane proteins which serve as membrane anchors for the catalytic subunits (5, 16).

The fine-structure genetic map of *glpT* has been refined in this work. Additional mutations have been mapped, including those carried by recombinant plasmids. The order of the amber mutations correlates with the sizes of the amber fragments synthesized in vitro (15) and provides additional evidence that the proposed direction of transcription of *glpT* on the linkage map of *E. coli* is correct. It should be possible to refine the map even further by the isolation of additional point mutations and specialized transducing phages.

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