Journal of Bacteriology, Jan. 1990, p. 424–430
0021-9193/90/010424-07$02.00/0
Copyright © 1990, American Society for Microbiology
Vol. 172, No. 1

Glycerol Facilitator of *Escherichia coli*: Cloning of *glpF* and Identification of the *glpF* Product

GAYE SWEET,[1]* CHRISTINE GANDOR,[1]† RALF VOEGELE,[1] NICOLA WITTEKINDT,[1]† JOACHIM BEUERLE,[1] VERONICA TRUNIGER,[1] E. C. C. LIN,[2] AND WINFRIED BOOS[1]

Department of Biology, University of Konstanz, D-7750 Konstanz, Federal Republic of Germany,[1] and Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115[2]

Received 26 May 1989/Accepted 2 October 1989

The glycerol facilitator is known as the only example of a transport protein that catalyzes facilitated diffusion across the *Escherichia coli* inner membrane. Here we show that the gene encoding the facilitator, *glpF*, is the first gene in an operon with *glpK*, encoding glycerol kinase, at 88 min on the *E. coli* chromosome. The operon is transcribed counterclockwise. We cloned the *glpF* gene, demonstrated that it complemented a chromosomal glycerol transport-minus mutation, and identified the gene product. The GlpF protein appeared in the membrane fraction of plasmid-bearing strains and had an apparent M_r of 25,000.

Glycerol uptake is commonly cited as the only example of transport by facilitated diffusion in *Escherichia coli*. It was recently suggested that there is also a propanediol facilitator (45). Glycerol, like other small uncharged molecules, can enter the cytoplasm by passive diffusion. Nevertheless, it was shown that uptake of glycerol is induced by glycerol or *sn*-glycerol-3-phosphate (G3P), repressed by growth in the presence of glucose, and constitutive in a *glp* regulon repressor mutant, evidence for a transport protein (39). Because of the membrane permeability of glycerol, if actively accumulated in the cytoplasm it would then be free to move down its concentration gradient, out of the cell. Instead, cytoplasmic glycerol is phosphorylated by glycerol kinase and thus trapped as G3P inside the cell (15). It was reported that nonmetabolizable polyhydric alcohols, such as ribitol and erythritol, are substrates of the glycerol facilitator and that transport is independent of phosphorylation, since these are not glycerol kinase substrates (16). Thus, the mechanism of glycerol transport is different from that of group translocation, where substrate enters the cytoplasm in a modified form. The glycerol facilitator was described as a channel in the inner membrane, allowing passage of polyhydric alcohols as well as unrelated small molecules like urea and glycine, but excluding charged molecules such as G3P and dihydroxyacetonephosphate (16).

It was reported that *glpF*, the gene encoding the glycerol facilitator, is in an operon with *glpK* (3, 9), the gene for glycerol kinase, and that *glpK* is promoter proximal (22). This operon, at 88 min on the *E. coli* chromosome (2), belongs to the *glp* regulon (reviewed in references 21 and 23). The proteins encoded by the *glp* regulon participate in uptake and metabolism of glycerol, G3P, and glycerophosphorylphosphodiesters. The *glp* genes are under common negative control, exerted by the product of the *glpR* gene (8, 40).

We set out to identify and characterize the glycerol facilitator, thought to mediate the simplest of *E. coli* transport processes. We established that *glpF* is the promoter-proximal gene in an operon with *glpK* and is transcribed counterclockwise. We have cloned *glpF* and identified its product as a membrane protein with an apparent M_r of 25,000.

* Corresponding author.
† Present address: Institute for Biotechnology, ETH-Hoenggerberg, CH-8093 Zurich, Switzerland.

MATERIALS AND METHODS

Bacterial strains, phages, and plasmids. Table 1 contains descriptions of the bacterial strains, phages, and plasmids used in this study. P1 transductions were performed by the method of Miller (28). When appropriate, XG (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), 20 μg/ml, was used to screen for the *lac* phenotype, and IPTG (isopropyl-β-D-thiogalactoside), 0.1 mM, was used for induction (both obtained from Boehringer). The *rha*::Tn*10* insertion in strain VT2 was obtained by transducing GD202 with a P1 lysate from a pool of random transposon Tn*10* insertions in MC4100 (created as described in reference 44), selecting for $glpK^+$. Strain GD229 was constructed by introducing a *glpR* mutation into GD173 by a method which will be the subject of a separate communication (Sweet and Boos, manuscript in preparation).

DNA methods were from Maniatis et al. (26) and Silhavy et al. (41).

λTn*phoA* (14) was used to create Tn*phoA* insertions into plasmids. After making the hop (as described in reference 14), plasmid DNA was isolated and retransformed, selecting for ampicillin (Ap^r) and kanamycin (Km^r) resistance. The *phoA* phenotype was detected on plates with XP (5-bromo-4-chloro-3-indolylphosphate), 20 μg/ml (Boehringer).

Growth. For genetic constructions or DNA preparation, bacterial strains were routinely grown in LB medium (28), and for glycerol transport or kinase measurements they were grown in minimal medium A (MMA) (28), containing 0.4% Casamino Acids (Difco Laboratories), with 5 mM G3P (10 mM D,L-G3P; Sigma Chemical Co.) for induction of the *glp* regulon when appropriate. Other carbon sources, where indicated, were present at 10 mM. When necessary, ampicillin was present at 100 μg/ml, tetracycline was present at 10 μg/ml, and kanamycin was present at 100 μg/ml, in minimal medium or at twice these concentrations in rich medium.

Glycerol transport assays. The rate of glycerol uptake was measured in a conventional transport assay with [U-^{14}C] glycerol (165.8 mCi/mmol; Amersham) present at 0.10 μM, cells at 10^8 per ml, in MMA. For screening the glycerol transport phenotype of large numbers of transductants or

TABLE 1. Bacterial strains, phages, and plasmids

Strain, phage, or plasmid	Description	Construction, source, or reference
E. coli K-12 derivatives		
7141	MC4100 *argE*::Tn*10*	J. Beckwith
BW11656	*recA*::*cat lac-169 pho-510 hsrR514*	B. Wanner
GD2	MC4100 Φ(*glpK*::*lacZ*) *zih-730*::Tn*10 glpR*	40
GD32	MC4100 Φ(*glpK*::*lacZ*) *glpR*	P1 GD2→TS100[a]
GD173	MC4100 *glpF*	Tc^s derivative of RJ70[b]
GD182	MC4100 *glpF recA*::*cat*	P1 BW11656→GD173
GD189	MC4100 *metB1*	P1 RhaD62→RJ70
GD192	MC4100 *glpF*::Tn*10 glpK*(Con)	Glycerol$^+$ RJ70[b]
GD202	MC4100 *glpK*	P1 Lin4→GD189
GD229	MC4100 *glpF glpR*	This study
GD235	JM103 *argE*::Tn*10*	P1 7141→JM103
GD236	JM103 *glpF*	P1 GD173→GD235
GD244	MC4100 *glpF*::Tn*10* Φ(*glpK*::*lacZ*) *glpR*	P1 RJ70→GD32
GD246	MC4100 *glpF*::Tn*10* Φ(*glpK*::*lacZ*) *pcnB80* Δ*rbs7*	P1 GD244→MRi80
HSK42	MC4100 *polA*	37
JM103	Δ(*lac-pro*) *thi strA supE endA sbcB15 hsdR4* (F' *traD36 proAB lacI^q* Δ*lacZM15*)	27
Lin4	*glpK* Δ*phoA* λ$^+$	19
MC4100	F$^-$ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 deoC1 ptsF25 rbsR*	6
MRi80	MC4100 *pcnB80* Δ*rbs7*	24
NK5587	F$^-$ Δ(*lac-pro*) *thiA rha trkA trkB* (F' *lacZ lacY*::Tn9)	N. Kleckner
RhaD62	*rhaD62 metB1* F$^+$ revertant of Hfr P72	33
RJ70	MC4100 *glpF*::Tn*10*	R. Jin
TL100	MC4100 *zih-730*::Tn*10*	T. Larson
TS100	MC4100 *glpR*	T. Silhavy
VT2	MC4100 *rha*::Tn*10*	This study
Phages		
λ*plac*Mu1	Mu cts62 *ner*$^+$A' *'ara'* Mu *S' 'lacZ lacY*$^+$ *lacA' imm*λ	5
λTn*phoA*	λ *b221 cI857 rex*::Tn*phoA*	14
mGP1-2	T7 gene 1 in M13mp8	S. Tabor
Plasmids		
pACYC184	Ap^r Cm^r	7
pBR322	Ap^r Tc^r	4
pGJ1	*glpK* (2.8-kb *Hin*dIII fragment) in pBR322, Ap^r	This study[c]
pK3	*glpK* (9.8-kb *Eco*RI fragment) in pACYC184, Tc^r	This study[c]
pT7-6	T7-Ø10 Ap^r	S. Tabor

[a] The donor and recipient strains used for construction by P1 transduction are indicated.
[b] See Table 2.
[c] See Fig. 2.

transformants, the transport assay was simplified. A 50-μl portion of an overnight culture was added to 500 μl of [^{14}C]glycerol, 0.11 μM in MMA (0.015 μCi/ml), and after 1 min of incubation, 500 μl was vacuum filtered through a membrane filter (0.45-μm pore size; Millipore), followed by an MMA wash and scintillation counting. For these transport measurements, strains must be *glpK*$^+$ for conversion of transported [^{14}C]glycerol to [^{14}C]G3P.

Visualization of plasmid-encoded proteins. After labeling with L-[^{35}S]methionine (Amersham; in vivo cell labeling grade; 1,331 Ci/mmol), cells were spheroplasted and osmotically lysed, and the membranes were pelleted at 100,000 × *g* for 1 h (34). Soluble proteins were precipitated with trichloroacetic acid, and membrane proteins were precipitated with acetone at −20°C. The pellets were solubilized in sample buffer at 37°C for 1 h and subjected to sodium dodecyl sulfate (SDS)-polyacrylamide (12.5%) gel electrophoresis (20), followed by autoradiography. Molecular weight standard proteins (SDS-7) were from Sigma.

RESULTS

***glpF* mutants.** A strain, RJ70 (isolated by R. Z. Jin), thought to contain a Tn*10* insertion in *glpK*, was instrumental in elucidating the organization of the *glpFK* operon and allowed isolation of useful *glpF* mutants with different levels of *glpK* expression. We observed that RJ70 grew very slowly on glycerol, indicating that the Tn*10* cannot be in *glpK*, since such an insertion would preclude growth on glycerol. We assayed glycerol kinase and found that it was expressed constitutively at a subbasal level (Table 2). Glycerol uptake measurements revealed that RJ70 had a glycerol transport-minus phenotype even after transformation with the *glpK* plasmid pGJ1. This suggested that the Tn*10* insertion was not in *glpK* but in an upstream transport gene, preventing expression of *glpK* from its own promoter. The low-level expression of kinase is presumably from the pOUT promoter of IS*10* (42). From RJ70 we isolated spontaneous derivatives exhibiting wild-type growth on 10 mM glycerol. These remained transport minus but had high constitutive levels of glycerol kinase activity (Table 2), perhaps the result of up-mutations in pOUT. A Tc^s, glycerol-plus derivative, GD173, isolated as described in reference 25, was still glycerol transport minus but had wild-type kinase activity. This polarity relief could be the result of nearly precise excision of the *glpF*::Tn*10*, leaving a small amount of Tn*10* DNA in the gene which was originally disrupted by the Tn*10*,

TABLE 2. Properties of *glpF* mutants

Strain	Tetracycline phenotype[a]	Growth on glycerol	Glycerol transport[b]	Glycerol kinase activity[c]	
				Uninduced	Induced
RJ70 (*glpF*::Tn*10*)	R	Slight	–	0.71	0.78
Glycerol[+] derivatives[d]	R	+	–	8.9	8.2
	S	+	–	24	19
	R[e]	+	–	20	16
GD173 (*glpF glpK*[+])	S[f]	+	–	1.7	17
MC4100 (wild type)	S	+	+	1.7	22

[a] R, Resistant; S, sensitive.
[b] [^{14}C]glycerol transport was measured in induced cells by the standard assay. RJ70 was first transformed with pGJ1.
[c] Glycerol kinase activity (in nanomoles of glycerol per minute per milligram of protein) was assayed (35) in sonicated cell extracts from uninduced or G3P-induced cells, after centrifugation for 1 h at 100,000 × *g* to pellet the membranes. Protein concentration (in milligrams per milliliter) was estimated by using the formula $1.45\ A_{280} - 0.74\ A_{260}$ (18).
[d] These strains were isolated from RJ70 as spontaneously growing faster on 10 mM glycerol.
[e] This strain was later named GD192.
[f] Selection was for Tc^s (25).

but allowing expression of the downstream *glpK* gene from the operon promoter (36).

We also isolated seven insertion mutants with glycerol-minus phenotypes. Strain GD189 was transduced with a P1 lysate from a cell population with random mini-*kan* insertions (44), selecting for *met*$^+$, and screening the Km^r transductants for glycerol defects. Two glycerol-minus transductants were transport minus and kinase minus, and five were transport plus but kinase minus. Thus, insertions in *glpF* are polar on *glpK*, whereas insertions in the distal *glpK* gene have no effect on expression of *glpF*. These results confirmed the *glpFK* operon organization (Fig. 1).

Cloning of *glpF*. A *glpF* mutation causes no clearly discernable growth defect at glycerol concentrations sufficient for colony formation, whereas a *glpK* mutant cannot grow on glycerol. We therefore used a *glpF glpK* mutant as recipient for the cloning of *glpF*, allowing direct selection of *glpK*$^+$. Strain GD246 was transformed with a plasmid library constructed by ligating *Sau*3A partially digested *E. coli* chromosomal fragments (6 to 20 kilobases [kb]) into the *Bam*HI site of pBR322, selecting for growth on glycerol in the presence of ampicillin. Transformants were screened for complementation of the chromosomal *glpF* mutation by the simplified glycerol transport assay. Transport-positive clones were only obtained after a *pcnB* mutation was introduced into the host strain (GD246) to reduce the plasmid copy number (24).

We had previously cloned *glpK* (unpublished) from a λgt7 *E. coli Eco*RI library (10). Our restriction map of the *glpK* plasmid was useful in the subcloning of *glpF*. After subcloning, it was no longer necessary to have a *pcn* mutation in the chromosome, indicating that in the original clones it was not *glpF* but the product of some other gene, distal to *glpK*, which was lethal in high doses.

Of the original plasmids that complemented *glpK* and *glpF*, pGD3 contained the shortest length of DNA upstream from *glpK* and so was chosen for subcloning of *glpF*. pGD3 (Fig. 2) was first digested with *Bst*EII, followed by conversion to blunt ends with the Klenow enzyme. This linearized plasmid was then digested with *Pvu*I, followed by blind ligation into pBR322 that had been cut with *Pvu*I and *Eco*RV. The DNA was transformed into GD173, selecting for Ap^r. The Tc^s transformants were screened for complementation of the chromosomal *glpF* mutation. We thus obtained pGC1 (6.5 kb), which carries the *glpF* gene on a 2.0-kb *Pvu*I-*Bst*EII (blunt) DNA fragment. Since the *Bst*EII site is located early in the *glpK* gene, pGC1 did not complement a *glpK* mutation (in GD202).

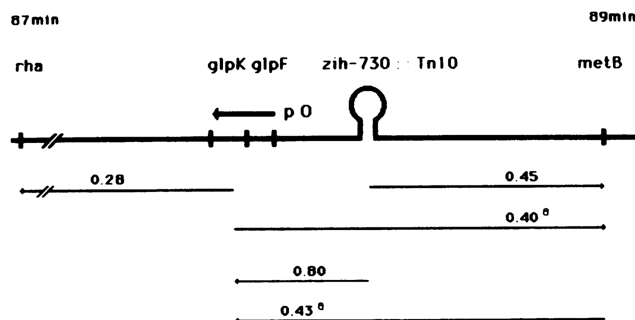

FIG. 1. Map position and direction of transcription of the *glpFK* operon. The arrow above the map indicates the counterclockwise direction of transcription of the *glpFK* operon. The position of *zih-730*::Tn*10* with respect to *glpK* and *metB* was determined by P1 transduction. The arrowheads below the map indicate the selected marker, and the numbers show the P1 cotransduction frequencies. [a]All transductants that received the distal marker also received the proximal marker from the donor.

To verify that we had cloned DNA from the *glpFK* region of the chromosome rather than another gene (with a similar restriction map) whose product complements the glycerol transport defect, we transformed a *polA* strain, HSK42 (37), with pGC1, selecting for Ap^r. The *polA* mutation precludes autonomous plasmid replication, and therefore transformants have the plasmid integrated into the chromosome over homologous recombination, the homology provided by the cloned DNA. We transduced such a *polA*(pGC1) transformant with P1 lysates from strains RJ70, TL100, and VT2, selecting for Tc^r and screening for Ap^r. We obtained the following cotransduction frequencies of the integrated plasmid with the indicated insertions: *glpF*::Tn*10*, 100%; *zih-730*::Tn*10*, 76%; and *rha*::Tn*10*, 20%. This confirmed that we had indeed cloned the *glpF* gene.

We further subcloned the *glpF* gene, making use of an *Nru*I site present in the *glpK* sequence (30). pGC1 was digested with *Nru*I and *Pvu*II and then religated to yield pGC2 (4.2 kb), with a 1.5-kb chromosomal insert containing *glpF*. pGC2 is presumably present in significantly higher copy number than pGC1, since removal of the *Pvu*II-*Bam*HI region of pBR322 has been reported to increase plasmid copy number (29).

Direction of transcription of the *glpFK* operon. Active *phoA* fusions to *glpF* were used to confirm the orientation of *glpF* on pGC2. Insertion plasmids were digested with *Dra*I. There is a *Dra*I site early in *phoA* and one in *bla*. Analysis of the restriction fragments clearly showed that the *glpF* promoter lies adjacent to the *tet'* gene of pBR322. *glpF* and *glpK* are transcribed in the same direction.

We determined the direction of transcription of the *glpFK* operon on the *E. coli* chromosome with respect to a nearby Tn*10* insertion (as described in reference 13). Strain GD2 has a chromosomal *glpK-lacZ* fusion that is 67% cotransducible with *zih-730*::Tn*10*. A *lacY*::Tn*9* insertion was transferred by P1 transduction from NK5587 to the *lacY* gene that is present on the λp*lac*Mu1 phage inserted in *glpK* in GD2. A P1 lysate was prepared and used to transduce GD32, which has the same *glpK-lacZ* fusion as GD2 but not the Tn*10*. Of the Tc^r transductants, 61% were also chloramphenicol resis-

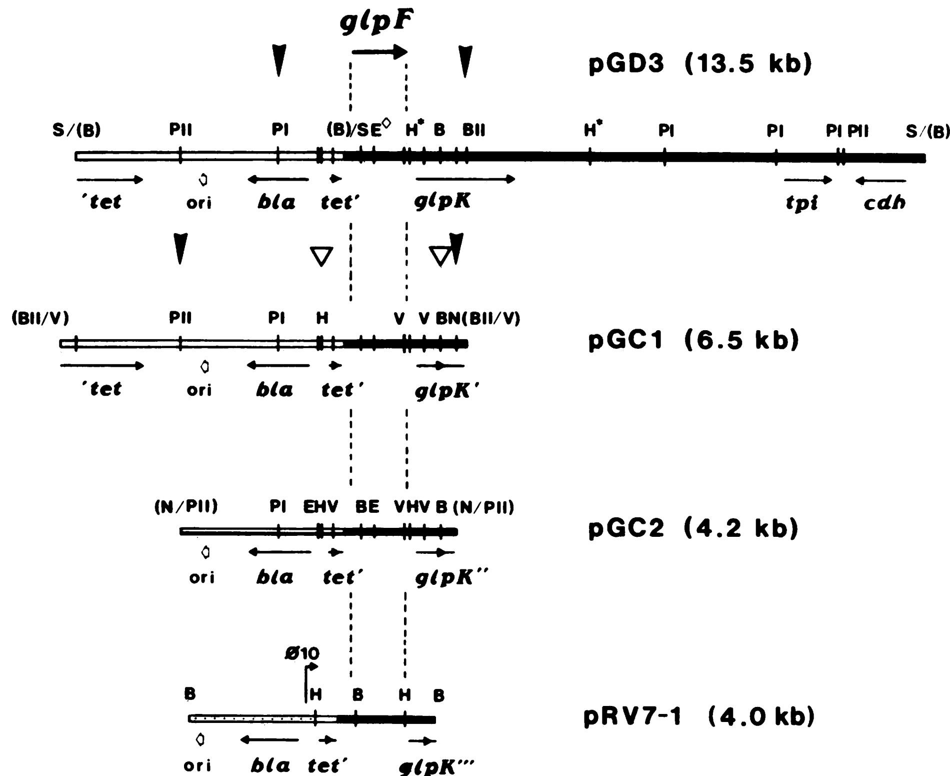

FIG. 2. Cloning of *glpF*. The *glpK*$^+$ *glpF*$^+$ plasmid pGD3 was the starting material for the subcloning of the *glpF* gene. Chromosomal DNA is shown as a black bar, and the vector pBR322 DNA is shown as a white bar. From comparison of restriction maps, pGD3 must also contain the genes *tpi*, encoding triosephosphate isomerase (31), and *cdh*, encoding CDP-diglyceride hydrolase (17). pBR322 genes are also shown. Arrows indicate directions of transcription. Restriction sites: B, *Bam*HI; BII, *Bst*EII; E, *Eco*RI; H, *Hin*dIII; N, *Nru*I; PI, *Pvu*I; PII, *Pvu*II; S, *Sau*3A (only the sites cut during the partial digestion of the chromosome are shown); V, *Eco*RV. Large solid arrowheads indicate the restriction sites used for subcloning plasmids below (open arrowheads show sites used to construct pRV7-1). Restriction sites shown in parentheses were no longer cleavable after ligation. It is likely that additional restriction sites for these enzymes are present beyond the *glpFK* operon, but we did not map them. Not all restriction sites are labeled, but none have been lost in the subcloning of *glpF*. In pRV7-1, the vector (dotted line) was pT7-6, carrying the T7-Ø10 promoter. The ◇ indicates one *Eco*RI end of the 9.8-kb insert in pK3, where the other *Eco*RI site must lie in *pfk*, encoding phosphofructokinase (1). The * indicates the 2.8-kb *Hin*dIII fragment present in the subcloned *glpK* plasmid pGJ1.

tant, similar to the cotransduction frequency of the *lac*$^+$ phenotype with the selected *zih-730*::Tn*10*. This indicated that the *glpFK* promoter is close to the Tn*10* and thus that the transcription direction was counterclockwise (Fig. 1). The opposite direction of transcription would have resulted in a dramatic reduction in cotransduction frequency due to the length of the λ DNA that would then have been present between the Tn*10* and the insertion in *lac*.

***glpF* plasmid-directed glycerol uptake.** That pGC1 and pGC2 in a *glpF* mutant confer the ability to transport glycerol is shown in Fig. 3A. GD173 carrying pBR322 was glycerol transport minus. The *glpF* plasmids complemented the transport defect. Transport increased with increasing numbers of copies of *glpF*. Both plasmid-bearing strains transported glycerol better than the strain with only chromosomal *glpF*$^+$ (MC4100; 135 pmol/min per 10^9 cells). The higher transport rate in the pGC2-bearing strain, 300 versus 200 pmol/min per 10^9 cells with pGC1, reflects the expected difference in plasmid copy number.

Testing for the inducibility of plasmid-encoded glycerol transport is complicated by coinduction of glycerol kinase. To circumvent this problem, we measured transport in a strain whose *glpK* expression is independent of *glp* control and constitutive (GD192). As can be seen in Fig. 3B, the plasmid-encoded transport was less in cells grown on glucose (235 pmol/min per 10^9 cells) than in cells grown on maltose (420 pmol/min per 10^9 cells), which exerts no catabolite repression (reviewed in reference 31). Growth on G3P, the inducer of the *glp* regulon (18), did not result in a significant increase in transport over the rate in maltose-grown cells. That expression of the cloned *glpF* gene is catabolite sensitive, as reported for the *glpFK* operon (12), suggests that it is expressed from its own promoter and not from the *tet* promoter that precedes it.

Additional evidence that the cloned *glpF* gene is preceded by its own promoter is that when pGC1 or pGC2 was present in a *glpR* mutant, in which *glp* genes are expressed constitutively, the strains grew very slowly. We frequently observed faster-growing cultures that invariably exhibited reduced or no glycerol transport, presumably the result of chromosomal mutations.

Identification of the *glpF* gene product. To detect the plasmid-encoded GlpF protein, it was necessary to examine cell fractions. For this purpose we prepared maxicells (38) by UV-irradiating a chromosomal *glpF recA* strain (GD182) carrying pBR322 or pGC2 (or derivatives). Shown in Fig. 4 is an autoradiogram of membrane proteins separated by polyacrylamide gel electrophoresis. A faint band at 25,000 appar-

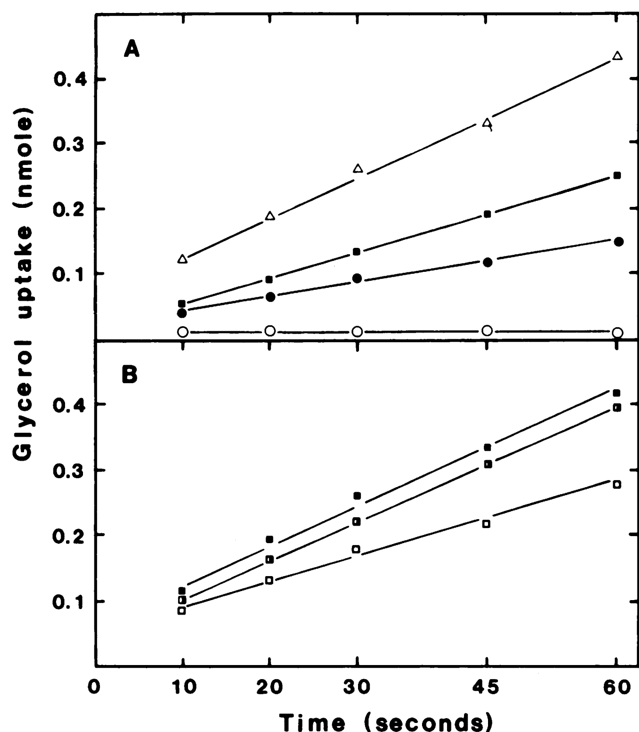

FIG. 3. Dependence of [^{14}C]glycerol transport on the cloned *glpF* gene. (A) Glycerol transport rates increase with increasing copies of *glpF*. Symbols: ○, none [GD173(pBR322)]; ●, chromosomal *glpF*$^{+}$ [MC4100(pBR322)]; ■, GD173(pGC1); △, GD173 (pGC2). Strains were induced by growth in the presence of G3P. (B) Glycerol transport rates in response to regulation of plasmid-encoded *glpF* expression. Uptake was measured in GD192(pGC1), in which glycerol kinase expression is constitutive. Cells were grown on glucose (□), maltose (◧), or G3P (■).

ent M_r corresponded to the GlpF protein. This band did not occur in the pBR322 control but was expressed from pGC2, both uninduced and induced by growth in the presence of G3P. The pBR322 *tet* gene was disrupted by the cloning of *glpF*, and therefore the Tet protein appeared only in the vector lane. Absolute assignment of the 25,000-M_r band to GlpF came from analysis of Tn*phoA* insertions in pGC2. An insertion that abolished the plasmid-encoded glycerol transport activity also resulted in disappearance of the GlpF band (lane −), whereas a transport-plus insertion plasmid still expressed the 25,000-M_r band (lane +). The *phoA* fusion plasmids were previously deleted with *Xho*I to remove the neomycin resistance protein that interfered with the detection of GlpF.

Overexpression of *glpF*. We cloned the *glpF* gene behind the T7-Ø10 promoter. pT7-6 was digested with *Bam*HI and *Hin*dIII, and pGC1 was partially digested with the same two enzymes, followed by blind ligation. The ligation mixture was transformed into GD173, selecting for Ap^{r} and screening for glycerol transport. This yielded pRV7-1 (4.0 kb; Fig. 2).

A more convincing demonstration of the product of *glpF* is presented in Fig. 5. Here, expression was from the T7 promoter. The GlpF band, at 25,000 M_r, was present primarily in the membrane fraction, not in the cytoplasm, and little in the periplasmic fraction. The GlpF in the periplasm (spheroplast supernatant) could represent cell lysis caused by overexpression. It is noteworthy that truncated glycerol kinase appeared with GlpF in the membrane and periplasmic

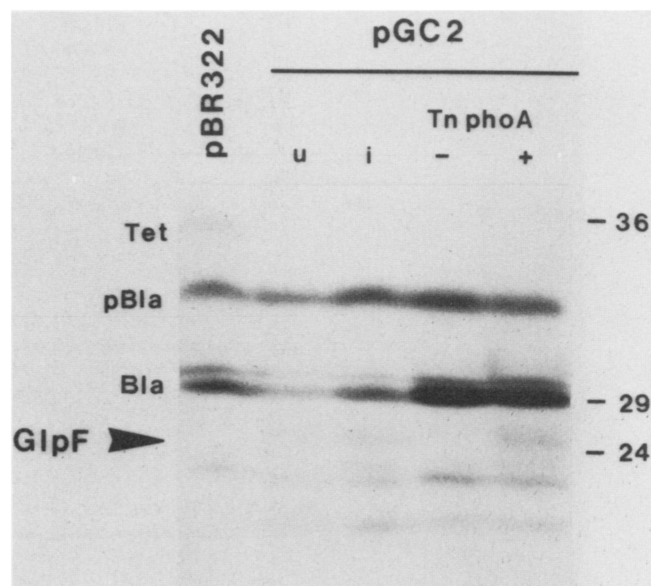

FIG. 4. *glpF* gene product. Membrane fractions were prepared from cells bearing pBR322 or pGC2. Lane u, Uninduced; lane i, induced with G3P. Tn*phoA* insertion: lane −, insertion in pGC2 that inactivated plasmid-encoded glycerol transport; lane +, insertion in pGC2 without effect on glycerol transport activity. Plasmid-encoded proteins were labeled in maxicells with [^{35}S]methionine, followed by cell fractionation, SDS-polyacrylamide gel electrophoresis, and autoradiography. The molecular masses of standard proteins are indicated on the right (in kilodaltons). pBR322-encoded proteins are shown on the left. The 25,000-M_r GlpF band is indicated with an arrowhead.

fractions rather than in the cytoplasm, where it was expected. We also observed intact glycerol kinase, expressed from pK3, in the membrane fraction, in contrast to triosephosphate isomerase, which was found only in the cytoplasmic fraction (data not shown). GlpK is posttranslationally

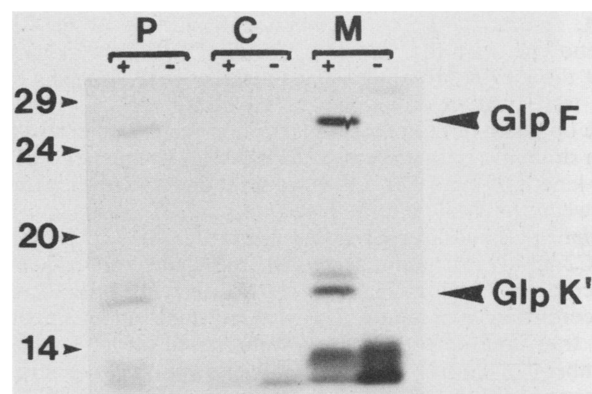

FIG. 5. *glpF* expressed from the T7-Ø10 promoter. Autoradiogram of [^{35}S]methionine-labeled membrane proteins from GD236 (pRV7-1) (lanes +) or GD236(pT7-6) as a negative control (lanes −). Fractions: P, periplasmic; C, cytoplasmic; M, membrane. The bands corresponding to GlpF (25,000 M_r) and the truncated GlpK (18,000 M_r) are indicated with arrowheads. Standard protein molecular masses are indicated on the left (in kilodaltons). Labeling was performed for 10 min after infection with mGP1-2, 30 min of induction with IPTG (2.5 mM), and 30 min of rifampin (200 μg/ml) treatment.

processed (30), so presumably the truncated GlpK polypeptide occurs as two bands because removal of the N-terminal methionine is incomplete.

DISCUSSION

We have established that *glpF* is in an operon with *glpK*. Characterization of insertion mutants demonstrated the promoter-proximal position of *glpF*. The operon is transcribed counterclockwise.

We have cloned the *glpF* gene, on a 1.5-kb DNA fragment from the 88-min region of the *E. coli* chromosome. Glycerol transport rates in induced, plasmid-bearing strains increased with *glpF* plasmid copy number. We are currently investigating the nature of the transport catalyzed by GlpF.

The *glpF* gene has most likely been cloned with its own promoter. We were able to demonstrate catabolite repression of glycerol transport in a strain in which the expression of *glpK* is uncoupled from its own *glp* promoter. That cells grown on G3P, for induction of plasmid-borne *glpF*, did not exhibit higher transport rates than did maltose-grown cells might indicate titration of the GlpR repressor protein. It could also be that the amount of constitutively expressed glycerol kinase is limiting. Proof of the presence of the *glpFK* operon promoter on the cloned DNA awaits completion of sequence determination and mRNA mapping.

GlpF, expressed in maxicells or from the T7 promoter, was identified as a membrane protein with an apparent M_r of 25,000. This is presumably an underestimate, since membrane proteins exhibit lower M_r on SDS gels, probably due to the binding of more SDS by the abundent hydrophobic amino acids (11). As a transport protein, GlpF is expected to span the *E. coli* inner membrane. This was confirmed by the isolation of active protein fusions to periplasmic alkaline phosphatase and cytoplasmic β-galactosidase (not shown). The detection of membrane-associated glycerol kinase supports previous suggestions that GlpF and GlpK interact physically to allow better substrate discrimination and rate control by the cell (22). Purification of GlpF is in progress.

ACKNOWLEDGMENTS

We thank J. Beckwith, E. Bremer, N. Kleckner, C. Manoil, S. Tabor, and B. Wanner for bacterial strains, phages, and plasmids, D. Boyd for the Tn*phoA* restriction map, M. Chen for the *E. coli* DNA library and critical reading of the manuscript, M. Ehrmann for a P1 lysate from a Tn*10* hop, T. Griffin for cells from a mini-*kan* hop, J. Richter for cheerful and skillful technical assistance, and M. Syvanen and J. Beckwith et al. for helpful discussions.

This work was supported by the Deutsche Forschungs Gemeinschaft (SFB 156) and by Public Health Service grant 5-R01-GM11983 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Albin, R., and P. M. Silverman.** 1984. Physical and genetic structure of the *glpK-cpxA* interval of the *Escherichia coli* K-12 chromosome. Mol. Gen. Genet. **197**:261–271.
2. **Bachmann, B. J.** 1987. Linkage map of *Escherichia coli* K-12, edition 7, p. 807–876. *In* F. C. Neidhardt (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. American Society for Microbiology, Washington, D.C.
3. **Berman-Kurtz, M., E. C. C. Lin, and D. P. Richey.** 1971. Promoter-like mutant with increased expression of the glycerol kinase operon of *Escherichia coli*. J. Bacteriol. **106**:724–731.
4. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multiple cloning system. Gene **2**:95–113.
5. **Bremer, E., T. J. Silhavy, J. M. Weismann, and G. M. Weinstock.** 1984. λ*plac*Mu: a transposable derivative of bacteriophage lambda for creating *lacZ* protein fusions in a single step. J. Bacteriol. **158**:1084–1093.
6. **Casadaban, M. J.** 1976. Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. J. Mol. Biol. **104**:541–555.
7. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**:1141–1156.
8. **Cozzarelli, N. R., W. B. Freedberg, and E. C. C. Lin.** 1968. Genetic control of the L-α-glycerolphosphate system in *Escherichia coli*. J. Bacteriol. **31**:371–387.
9. **Cozzarelli, N. R., and E. C. C. Lin.** 1966. Chromosomal location of the structural gene for glycerol kinase in *Escherichia coli*. J. Bacteriol. **91**:1763–1766.
10. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. **de Jong, W. W., A. Zweers, and L. H. Cohen.** 1978. Influence of single amino acid substitutions on electrophoretic mobility of sodium dodecyl sulfate-protein complexes. Biochem. Biophys. Res. Commun. **82**:532–539.
12. **Freedberg, W. B., and E. C. C. Lin.** 1973. Three kinds of controls affecting the expression of the *glp* regulon in *Escherichia coli*. J. Bacteriol. **115**:816–823.
13. **Freundlieb, S., and W. Boos.** 1986. α-Amylase of *Escherichia coli*, mapping and cloning of the structural gene, *malS*, and identification of its product as a periplasmic protein. J. Biol. Chem. **261**:2946–2953.
14. **Gutierrez, C., J. Barondess, C. Manoil, and J. Beckwith.** 1987. The use of transposon Tn*phoA* to detect genes for cell envelope proteins subject to a common regulatory stimulus. Analysis of osmotically regulated genes in *Escherichia coli*. J. Mol. Biol. **195**:289–297.
15. **Hayashi, S.-I., and E. C. C. Lin.** 1965. Capture of glycerol by cells of *Escherichia coli*. Biochim. Biophys. Acta **94**:479–487.
16. **Heller, K. B., E. C. C. Lin, and T. H. Wilson.** 1980. Substrate specificity and transport properties of the glycerol facilitator of *Escherichia coli*. J. Bacteriol. **144**:274–278.
17. **Icho, T., C. E. Bulawa, and C. R. H. Raetz.** 1986. Molecular cloning and sequencing of the gene for CDP-diglyceride hydrolase of *Escherichia coli*. J. Biol. Chem. **260**:12092–12098.
18. **Kalckar, H. M.** 1947. Differential spectrophotometry of purine compounds by means of specific enzymes. III. Studies of the enzymes of purine metabolism. J. Biol. Chem. **167**:461–475.
19. **Koch, J. P., S.-I. Hayashi, and E. C. C. Lin.** 1964. The control of the dissimilation of glycerol and L-α-glycerophosphate in *Escherichia coli*. J. Biol. Chem. **239**:3106–3108.
20. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
21. **Lin, E. C. C.** 1976. Glycerol dissimilation and its regulation in bacteria. Annu. Rev. Microbiol. **30**:535–578.
22. **Lin, E. C. C.** 1984. Glycerol utilization by facilitated diffusion coupled to phosphorylation in bacteria, p. 109–130. *In* E. Haber (ed.), The cell membrane. Plenum Publishing Corp., New York.
23. **Lin, E. C. C.** 1987. Dissimilatory pathways for sugars, polyols, and carboxylates, p. 244–284. *In* F. C. Neidhardt (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 1. American Society for Microbiology, Washington, D.C.
24. **Lopilato, J., S. Bortner, and J. Beckwith.** 1986. Mutations in a new chromosomal gene of *Escherichia coli* K-12, *pcnB*, reduce plasmid copy number of pBR322 and its derivatives. Mol. Gen. Genet. **205**:285–290.
25. **Maloy, S. R., and W. D. Nunn.** 1981. Selection for loss of tetracycline resistance by *Escherichia coli*. J. Bacteriol. **145**:1110–1112.
26. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

27. **Messing, J., R. Crea, and P. H. Seeburg.** 1981. A system for shotgun DNA sequencing. Nucleic Acids Res. **9:**309–321.
28. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Minton, N. P.** 1984. Improved plasmid vectors for the isolation of translational *lac* gene fusions. Gene **31:**269–273.
30. **Pettigrew, D. W., D.-P. Ma, C. A. Conrad, and J. R. Johnson.** 1988. *Escherichia coli* glycerol kinase. Cloning and sequencing of the *glpK* gene and the primary structure of the enzyme. J. Biol. Chem. **263:**135–139.
31. **Pichersky, E., L. D. Gottlieb, and J. F. Hess.** 1984. Nucleotide sequence of the triose phosphate isomerase gene of *Escherichia coli*. Mol. Gen. Genet. **195:**314–320.
32. **Postma, P. W., and J. W. Lengeler.** 1985. Phosphoenolpyruvate: carbohydrate phosphotransferase system of bacteria. Microbiol. Rev. **49:**232–269.
33. **Powers, J.** 1967. The L-rhamnose genetic system in *Escherichia coli*. Genetics **55:**557–568.
34. **Randall, L. L., and S. J. S. Hardy.** 1986. Correlation of competence for export with lack of tertiary structure of the mature species: a study *in vivo* of maltose-binding protein export in *Escherichia coli*. Cell **46:**921–928.
35. **Richey, D. P., and E. C. C. Lin.** 1972. Importance of facilitated diffusion for effective utilization of glycerol by *Escherichia coli*. J. Bacteriol. **112:**784–790.
36. **Ross, D. G., J. Swan, and N. Kleckner.** 1979. Nearly precise excision: a new type of DNA alteration associated with the translocatable element Tn*10*. Cell **16:**733–738.
37. **Saarilahti, H. T., and E. T. Palva.** 1985. *In vivo* transfer of chromosomal mutations onto multicopy plasmids using *polA* strains: cloning of an *ompR2* mutation in *Escherichia coli* K12. FEMS Lett. **26:**27–33.
38. **Sancar, A., A. M. Hack, and W. D. Rupp.** 1979. Simple method for identification of plasmid-coded proteins. J. Bacteriol. **137:**692–693.
39. **Sanno, Y., T. H. Wilson, and E. C. C. Lin.** 1968. Control of permeation to glycerol in cells of *Escherichia coli*. Biochem. Biophys. Res. Commun. **32:**344–349.
40. **Schweizer, H., W. Boos, and T. J. Larson.** 1985. Repressor for the *sn*-glycerol-3-phosphate regulon of *Escherichia coli* K-12: cloning of the *glpR* gene and identification of its product. J. Bacteriol. **161:**563–566.
41. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
42. **Simons, R. W., B. C. Hoopes, W. R. McClure, and N. Kleckner.** 1983. Three promoters near the termini of IS*10*: pIN, pOUT, and pIII. Cell **34:**673–682.
43. **Tabor, S., and C. C. Richardson.** 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled expression of specific genes. Proc. Natl. Acad. Sci. USA **82:**1074–1078.
44. **Way, J. C., M. A. Davis, D. Morisato, E. E. Roberts, and N. Kleckner.** 1984. New Tn*10* derivatives for transposon mutagenesis and for construction of *lacZ* operon fusions by transposition. Gene **32:**369–379.
45. **Zhu, Y., and E. C. C. Lin.** 1989. L-1,2-Propanediol exits more rapidly than L-lactaldehyde from *Escherichia coli*. J. Bacteriol. **171:**862–867.