JOURNAL OF BACTERIOLOGY, June 1982, p. 1164–1171
0021-9193/82/061164-08$02.00/0

Vol. 150, No. 3

Mapping of Two *ugp* Genes Coding for the *pho* Regulon-Dependent *sn*-Glycerol-3-Phosphate Transport System of *Escherichia coli*

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Received 3 August 1981/Accepted 1 February 1982

Two genes, *ugpA* and *ugpB*, coding for a binding protein-dependent *sn*-glycerol-3-phosphate transport system, were mapped at 75.3 min on the *Escherichia coli* chromosome. A Tn*10* insertion in *ugpA* resulted in loss of transport activity but still allowed the synthesis of the *sn*-glycerol-3-phosphate-binding protein. This Tn*10* insertion was found to be linked by P1 transduction to *pit*, *aroB*, *malA*, *asd*, and *livH* with 2.5, 2.8, 25, 63.5, and 82% cotransduction frequency. An insertion of Mud (Amp^r *lac*) in *ugpB* resulted in the loss of the binding protein. *ugpB* is closely linked to *ugpA*. It is either the structural gene for the binding protein or located proximal to it. The analysis of the crosses allowed the ordering of the markers in the clockwise direction as follows: *aroB*, *malA*, *asd*, *ugpA*, *ugpB*, *livH*, *pit*.

Escherichia coli contains two transport systems for *sn*-glycerol-3-phosphate (G3P). One, coded for by the *glpT* region at 48 min on the *E. coli* chromosome, is under the control of the *glp* regulon (9). Thus, it is induced by G3P and constitutive in mutants missing a functional regulator gene (*glpR*). By transporting G3P, this system can supply the sole source of carbon as well as phosphate. In contrast, the second, *ugp*-dependent system can supply only G3P as a sole source of phosphate, but not carbon (25). It is under the control of the *pho* regulon. Thus, the *ugp*-dependent G3P transport activity is present only after growth at limiting concentrations of P_i. In addition, it is constitutively synthesized in strains derepressed for alkaline phosphatase (3). So far, the *pho* regulon is known to comprise at least three different operons: *phoA*, coding for the periplasmic alkaline phosphatase (24) at 9 min; *phoE*, coding for the outer membrane pore protein PhoE (Ic, e, E) (28) at 6 min; and *pst phoS phoT*, coding for a binding protein-dependent P_i transport system (2, 29) at 83 min on the *E. coli* linkage map (4). To further characterize the *pho*-dependent G3P transport system, it was necessary to map the responsible genes and find their relation to the known operons of the *pho* regulon. The analysis was facilitated by the finding that 3,4-dihydroxybutyl-1-phosphonate (DHBP), a toxic analog of G3P (18), is specifically transported by the system and allows the isolation of mutants (*ugp*) as well as the screening of the Ugp phenotype (25). In addition, the use of the tetracycline resistance (Tc^r) transposon Tn*10* allowed the insertion of this selective marker in, as well as close to, the *ugp* genes. By applying these techniques (11, 16), we could establish that the *ugp* genes form a cluster that is separate from all other known *pho* genes.

Preliminary experiments using Hfr-mediated crosses indicated that the *ugp* region was located between *rpsL* (72 min) and *mtl* (80 min) on the *E. coli* linkage map (4). The present paper deals with genetic mapping by P1 transduction of *ugp* mutations in relation to the nearby markers *aroB*, *malA*, *asd*, *livH*, and *pit*.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Table 1 lists the strains used. Phage TC45 specific for protein PhoE (Ic, e, E) was originally obtained from U. Henning; phage λNK370 *c*I171::Tn*10* was obtained from N. Kleckner; and phage hy-2 was obtained from T. Silhavy.

Media. Minimal medium for growth of cultures was minimal medium A (22) or Tris medium (13) supplemented with 0.2% of the appropriate carbon source and 40 μg of the required amino acids per ml, except for D-leucine, which was used at 100 μg/ml (1). Thymine was present at 50 μg/ml, and vitamin B_1 was present at 10 μg/ml. LB or DYT medium (22) was used as the rich medium. R plates (22) were used for phage titering and lysate preparation. For preparing plates, all of the media listed above were solidified with 1.5% agar. All growth media were obtained from Difco Laboratories, Detroit, Mich.

Genetic manipulations. P1 transductions and Hfr

TABLE 1. Bacterial strains

Strain	Parent	Method of isolation	Known markers	Reference or source
SH23	CA77	Transduction of CA77 with P1 grown on CGSC 4934; selection for growth on β-glycerol-phosphate	Hfr Δ*lac phoR17*	This study
SH24	SH23	Transduction of SH23 with P1 grown on DL42	Hfr Δ*lac phoR17 gyrA* Δ*glpT596*	This study
SH34	AB1157 × SH54	Hfr cross, selection for ProA$^+$, screening for Ugp	*ugp-702 proA*$^+$	This study
SH41	SH34 × SH54	Hfr cross, selection for Xyl$^+$, screening for Ugp	*ugp-702 xyl*$^+$	This study
SH42	SH41	Transduction of SH41 with P1 grown on TS100 with random insertions of Tn*10*	*zhf-721*::Tn*10 ugp*$^+$; otherwise as SH41	This study
SH43	SH41	Transduction of SH41 with P1 grown on SH42	*zhf-721*::Tn*10 ugp-702*; otherwise as SH41	This study
SH44	SH41	Transduction of SH41 with P1 grown on SH42	*zhf-721*::Tn*10 proA*$^+$ *xyl*$^+$	This study
SH46	AE4107	Transduction of AE4107 with P1 grown on SH42	*zhf-721*::Tn*10*; otherwise as AE4107	This study
SH47	AE4107	Spontaneous *malT*$^+$	*malA*$^+$; otherwise as AE4107	This study
SH54	CGSC4934	Spontaneous DHBP resistance	Hfr *ugp-702*; otherwise as CGSC 4934	This study
SH101	LA3430	Growth on β-glycerolphosphate	Hfr *glpT phoR*	25
SH102	LA3430	Growth on β-glycerolphosphate	Hfr *glpT phoR*	25
SH115			Hfr *glpK* Δ*glpT gyrA phoA8 phoT ilv*::Tn*10*	25
SH123	AE4107	Growth on β-glycerolphosphate	*pho*^{ca}; otherwise as AE4107	This study
SH304	SH24	Insertion of Mud (Amp^r *lac*) from MAL103 in SH24, selection for Amp^r and DHBP^r	*ugp-701*::Mud (Amp^r *lac*); otherwise as SH24	This study
SH306	SH24	Insertion of Mud (Amp^r *lac*) from MAL103 in SH24, selection for Amp^r and DHBP^r	*ugpB-703*::Mud (Amp^r *lac*); otherwise as SH24; strain lacks the G3P-binding protein	This study
SH606	SH306	Spontaneous λ^r	*malT*; otherwise as SH306	This study
SH1200			Hfr *glpT ugpA704*::Tn*10 phoR*	25
SH1230	LA5415	Transduction of LA5415 with P1 grown on SH1200	*ugpA704*::Tn*10 aroB*	This study
SH1238	SH306	Transduction of SH306 with P1 grown on SH1200	*ugpA704*::Tn*10 ugpB*$^+$; otherwise as SH306	This study
SH1239	SH306	Transduction of SH306 with P1 grown on SH1200	*ugpA704*::Tn*10*; otherwise as SH306	This study
AE4107			*lstR leu6 malA1 xyl-7 mtl-2 argG6 hisA trp-81 strp-104 gyrA thy livH*::Mu	(1)
HS2050	MC4100		F$^-$ *rif*^{r} *metA malPQ*::Tn*5 araD139* Δ*lacU169 ptsF25 flbB*	H. Shuman
Hfr G6 ΔMD2			Hfr *hisA323* Δ(*bioH-gntR*) *araF*	31; M. Schwartz
Hfr G6 ΔMD3			Hfr *hisA323* Δ(*malA-gntR*) *araF*	31; M. Schwartz
Hfr G6 ΔMD18			Hfr *hisA323* Δ(*bioH-glpD*) *araF*	31; M. Schwartz
TS100	MC4100		F$^-$ *araD139* Δ*lacU169 thi relA rpsL glpR ptsF25 flbB*	25
10B5			HfrC *pst-2 pit-1 glpD3 glpR2 phoA8 tonA22 relA*	27
CGSC 4829			HfrC *phoA8 relA1 tonA22 pit-10 spoT1*	Coli Genetic Stock Center; B. Bachmann

Continued on following page

TABLE 1—*Continued*

Strain	Parent	Method of isolation	Known markers	Reference or source
CGSC 4934			HfrC *phoR17 tonA22 pit-10 spoT1*	Coli Genetic Stock Center; B. Bachmann
BW711			Δ*lacU169 thi rpsL aroB proC*::Tn*5* *ilv*::Tn*10*	B. Wanner
72			HfrC Δ(*glpR-malA*) *phoA8*	19
MAL103			F⁻ Mu *cts* d1 (Amp^r *lac*) Mu *cts* Δ(*proA,B-lacIPOZYA*)*XIII rpsL*	7
AB1157			F⁻ *thi-1 argE3 his-4 proA2 leu-6 thr-1 lacY1 galK2 ara-14 mtl-1 xyl-5 rpsL31 tsx-33 sup-37* λ⁻	12, 20
DL42			F⁻ *glpR* Δ*glpT596 gyrA thi* Δ*lacU169 araD139 ptsF25 flbB*	D. Ludtke
Hfr 3000 U482			HfrH *asd-1 thi-1 relA1 spoT1* λ⁻	15
CA77	Hfr 3000 × 74		Hfr *thi-1 relA1 spoT1* Δ(*lac*) DE3 λ⁻	S. Brenner

[a] *pho*^{c} designates constitutivity of the *pho* regulon of unknown nature.

crosses were done as described by Miller (22). Selection of tetracycline-resistant mutants obtained by P1 transduction of Tn*10* was done as described previously (25). Spontaneous tetracycline-susceptible (Tc^s) mutants were isolated by a recently described method (6) with further modification (21). Insertion of the tetracycline resistance transposon Tn*10* in the *ugp* genes was done by transducing *ugp*$^+$ strain SH101 with a P1 lysate grown on pooled transductants of strain TS100 with random Tn*10* insertions in the chromosome after infection with phage λNK370, selecting for Tc^r as previously described (16). About 30,000 Tc^r transductants were pooled in 50 ml of LB medium. One milliliter of this suspension was diluted into 50 ml of fresh LB medium (2.5×10^8 cells/ml) and aerated at 37°C to a cell density of 10^9 cells/ml. A portion of this suspension was diluted 100-fold (10^7 cells/ml) in 1 ml of minimal medium A, containing 0.2% glucose and 2.5 mM DHBP, and aerated at 37°C to yield a density of 10^9 cells/ml. This cycle was repeated once by diluting 10^4-fold (10^5 cells/ml) in 1 ml of the same medium. After growth overnight, the suspension was diluted in minimal medium A to 10^3 cells/ml and plated on nutrient broth plates containing 20 μg of tetracycline per ml. About 2,500 colonies were tested for constitutive alkaline phosphatase activity by spraying with 10 mg of *p*-nitrophenyl phosphate per ml in 1 M Tris-hydrochloride (pH 8.0). Only one colony was found possessing constitutive alkaline phosphatase that was negative in *ugp*-dependent G3P transport activity.

To insert Tn*10* close to *ugp*, the P1 lysate of pooled random Tn*10* insertions in TS100 (obtained from 2×10^4 independent insertions) was used to transduce strain SH54 (*ugp-702*) to Tc^r. Colonies were replica plated onto LB plates containing 20 μg of tetracycline per ml and 0.1 μM [*U*-^{14}C]G3P (153 mCi/mmol; New England Nuclear Corp., Boston, Mass.). After growth, the colonies were transferred to sterile filter paper. The filter was dried and subjected to autoradiography for 2 days. Dark colonies were purified and tested for *ugp*-dependent transport activity for G3P and cotransducibility of Tc^r with *ugp*$^+$.

Insertion of Mud (Amp *lac*) in the *ugp* genes was done as described (7), followed by two DHBP enrichment cycles as described above. The same DHBP enrichment procedure was also used to select spontaneous mutants in *ugp*. Sensitivity against bacteriophage TC45 (8) and phage hy-2 (5) was tested by cross-streaking on R plates (22).

Transport assays. *ugp*$^+$-dependent G3P transport activity was examined as previously described (25).

Preparation of osmotic shock proteins. To isolate periplasmic shock fluid, cells were grown overnight at 37°C in 400 ml of DYT medium (25). The osmotic shock procedure was done by the method of Neu and Heppei (23) with modifications as described (26). Proteins were analyzed by sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis according to Laemmli (17).

Immunodiffusion assay for the G3P-binding protein. To test for the presence of G3P-binding protein, periplasmic proteins were isolated by an EDTA-lysozyme spheroplast formation technique. One milliliter of an overnight culture in DYT medium was diluted with 9 ml of the same medium and grown under aeration for another 2 h. After harvesting, the cells were washed twice in minimal medium A. The pellet was suspended in 0.1 ml of 10 mM Tris-hydrochloride (pH 7.3) containing 20% sucrose and 10 mM EDTA. Lysozyme was added to a final concentration of 0.1 mg/ml, and the whole suspension was incubated for 1 h at room temperature. The cell suspension was centrifuged in an Eppendorf 5412 centrifuge for 5 min. The supernatant was removed and centrifuged for another 3 min, and a 10-μl portion was transferred to the outer well of an immunodiffusion plate prepared of 1% agarose, type II (Sigma Chemical Co., St. Louis, Mo.), in 50 mM diethylbarbituric acid (pH 8.6) containing 0.2% sodium azide. Rabbit antiserum against purified binding protein was placed in the center well. After incuba-

tion overnight at 37°C, the immunodiffusion plates were washed in 500 ml of 2% sodium chloride overnight. The precipitin bands were stained with 0.1% Coomassie brillant blue in methanol-water-acetic acid (20:17:3, by volume) and destained overnight in water-methanol-acetic acid (100:15:7, by volume).

Alkaline phosphatase activity. Alkaline phosphatase activity (13) in whole cells was determined as described previously (25).

RESULTS

Use of DHBP in the isolation of *ugp* mutants. As shown previously, the toxic G3P analog DHBP is transported by the *ugp*-dependent G3P transport system (25). Thus, from strains lacking the *glpT*-dependent G3P transport system but containing constitutive levels of the *ugp* system, mutants could be isolated that exhibited defects in this transport system. In this way, three mutants were isolated. SH54 is a spontaneous *ugp* mutant. It is constitutive for alkaline phosphatase (*phoR17*) and still synthesizes the G3P-binding protein as tested by cross-reactivity against specific antibodies. Its allele number is *ugp-702*. SH1200 also synthesizes the G3P-binding protein but was isolated after insertion of the tetracycline resistance transposon Tn*10* into *ugpA*. Its allele number is *ugpA704*::Tn*10*. The *pho* constitutivity of this strain is also due to a *phoR* mutation. P1 lysates grown on SH1200 transduced the Ugp^- phenotype 100% linked to Tc^r. Transport activity of G3P in SH1200 was very low in comparison to a fully constitutive strain. Transducing such a fully active strain with a lysate of SH1200 to Tc^r resulted in the same transport negativity as in SH1200 (Fig. 1). SH304 carries an insertion of the phage Mud (Amp^r *lac*) (7). The strain is Lac^+ and still synthesizes the G3P-binding protein. Its allele number is *ugp-701*::Mud (Amp^r *lac*). The *pho* constitutivity of this strain is due to a mutation in the *pst*-dependent phosphate transport system. A similar strain is SH306. The insertion of Mud (Amp^r *lac*) in this strain has resulted in the loss of G3P-binding protein. In addition, it is Lac^-. Since SH306 lacks the G3P-binding protein, the insertion is not in *ugpA*. It is either within the structural gene for the G3P-binding protein or in a gene proximal to it. Its allele number is *ugpB703*::Mud (Amp^r *lac*).

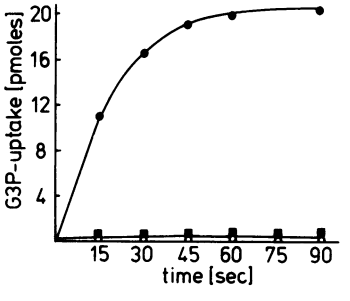

FIG. 1. Effect of the insertion of Tn*10* in different *ugp* mutants on the *ugp*-dependent transport activity. Strains were grown in minimal medium A with glucose as the carbon source to an optical density of 0.5 (578 nm), and transport was measured. The results are given as amount of G3P taken up per 200 μl of cell suspension (optical density of 0.5) at room temperature. Symbols: ●, ugp^+ strain SH101; ○, *ugpA704*::Tn*10* strain SH1200; ■, ugp^+ strain SH101 transduced to Tc^r with P1 grown on SH1200.

Mapping of *ugpA704*::Tn*10* with nearby markers. Table 2 contains the results of P1 transductions with lysates of strain SH1200 (*ugpA704*::Tn*10*) into different strains carrying the markers *livH*, *asd*, *glpD*, *malA*, *aroB*, and *pit*. Cotransduction frequencies of 82, 63.5, 25, 25, 2.8, and 2.5%, respectively, were found. To determine the relative order of *ugpA704*::Tn*10* to *malA* and *livH*, crosses 1 and 6 of Table 2 were analyzed as three-factor crosses in Table 3. From cross A, it was evident that *ugpA* was closer to *livH* than to *malA*. Cross B indicated the order *malA*, *ugpA*, *livH*. This order was further corroborated by a cross using a marker distal to *livH* (cross 4 in Table 4). The cotransduction frequencies of *ugpA* to *aroB*, *asd*, and *pit* together with their established positions on the *E. coli* linkage map (4) allowed the formulation of the following order: *rpsL*, *aroB*, *malA*, *glpD*, *asd*, *ugpA*, *livH*, *pit*. With a fix point for *malA* at 74.7 min (4) and using the formula of Wu (30), this analysis placed *ugpA* at 75.3 min.

During the genetic analysis of the *malA* region, deletions were isolated covering *malA* and *glpD* (15). Among these deletions, two also covered *asd* and *gntR*, whereas one did not (31). The test of these deletions for the *ugp*-dependent G3P transport activity revealed that all three strains contained the transport system. This demonstrated that *asd* and *gntR* (31) were counterclockwise to *ugp*. In addition, we observed that all three strains, two of which carried deletions extending from *malA* through *bioH*, were still sensitive for phage hy-2. Therefore, these deletions cannot cover *ompB*, which is necessary for the expression of the outer membrane protein *ompC* (14), the receptor for phage hy-2 (5). Thus, in contrast to the present position on the *E. coli* linkage map, *ompB* has to be located counterclockwise to *bioH*. The position of *ugp* to its nearby markers is given in Fig. 2.

Mapping of *zhf-721*::Tn*10* to nearby markers. Table 2 contains the results of P1 transductions with lysates of strains SH42 and SH43 (*zhf-721*::Tn*10*) with different strains carrying the markers *livH*, *ugpB703*::Mud (Amp^r *lac*), *pit*, and *malA(T)*. Cotransduction frequencies of 69, 64, 15, and 14%, respectively, were found.

TABLE 2. Transduction frequencies of loci in the *malA* region

Cross	Relevant genotype		Selected marker	Unselected marker	% with unselected marker
	Donor	Recipient			
1	SH1200 *ugpA704*::Tn*10*	AE4107 *livH*::Mu	Tc^r	$LivH^{+a}$	81.8 (409/500)
2	SH1200 *ugpA704*::Tn*10*	Hfr 3000 U482 *asd*	Tc^r	Asd^+	63.5 (127/200)
3	SH1200 *ugpA704*::Tn*10*	10B5 *glpD*	Tc^r	$GlpD^+$	25.4 (18/71)
4	SH1200 *ugpA704*::Tn*10*	HS2050 *malP,Q*::Tn*5*	$Tc^{r b}$	$MalA^+$	25.3 (43/170)
5	SH1200 *ugpA704*::Tn*10*	72 *malA*	Tc^r	$MalA^+$	25.3 (41/162)
6	SH1200 *ugpA704*::Tn*10*	AE4107 *malA1*	Tc^r	$MalA^+$	33.6 (168/500)
7	SH1200 *ugpA704*::Tn*10*	LA5415 *aroB*	Tc^r	$AroB^+$	3.1 (5/160)
8	SH1230 *ugpA704*::Tn*10 aroB*	72 *aroB*$^+$	Tc^r	$AroB^-$	2.5 (4/163)
9	SH1200 *ugpA704*::Tn*10*	CGSC 4829 *pit*	Tc^r	Pit^{+b}	2.5 (3/121)
10	SH44 *zhf-721*::Tn*10*	SH606 *ugpB703*::Mud (Amp^r *lac*)	Tc^r	Amp^s	72.0 (72/100)
11	SH43 *zhf-721*::Tn*10*	SH123 *livH*::Mu	Tc^r	$LivH^+$	63.5 (137/200)
12	SH43 *zhf-721*::Tn*10*	SH606 *ugpB703*::Mud (Amp^r *lac*)	Tc^r	Amp^s	57.0 (57/100)
13	SH43 *zhf-721*::Tn*10*	SH606 *malT*	Tc^r	$MalT^+$	18.0 (18/100)
14	SH43 *zhf-721*::Tn*10*	CGSC 4934 *pit*	Tc^r	Pit^+	17.8 (36/202)
15	SH42 *zhf-721*::Tn*10*	SH41 *pit*	Tc^r	Pit^+	12.1 (13/107)
16	SH43 *zhf-721*::Tn*10*	AE4107 *malA1*	Tc^r	$MalA^+$	10.0 (28/279)
17	SH43 *ugp-702 zhf-721*::Tn*10*	SH123	Tc^r	Ugp^{-c}	63.5 (127/200)

[a] *livH*$^+$ scored as ability to use 100 μg of D-leucine per ml as an L-leucine source.
[b] *pit*$^+$ scored as sensitivity to 10 mM arsenate on Tris medium supplemented with 1 mM P_i.
[c] *ugp* scored as ability to grow on minimal glucose plates in the presence of 2.5 mM DHBP.

To determine the relative order of these markers, three-factor crosses were done, selecting for tetracycline resistance. In the first cross with *livH* and *malA* as unselected markers, the rarest recombinant class was *livH mal*$^+$ (Table 4). Since cotransduction to *livH* was high, the Tn*10* insertion must be located outside *malA* and *livH*, in the clockwise direction *malA*, *livH*, *zhf-721*::Tn*10*. For the same reason, the Tn*10* insertion must be located outside the two markers *malA* and *ugpB703*::Mud (Amp^r *lac*) in the clockwise order *malA*, *ugpB703*::Mud (Amp^r *lac*), *zhf-721*::Tn*10* (crosses 2 and 3). In cross 4, the clockwise order *ugp-702*, *livH*, *zhf-721*::Tn*10* was determined. Thus, the Tn*10* insertion was distal to *livH*, and the clockwise order of these markers was *malA(T)*, *ugp*, *livH*, *zhf-721*::Tn*10*.

The gene coding for the G3P-binding protein is closely linked to *ugpA*. When strain SH1200

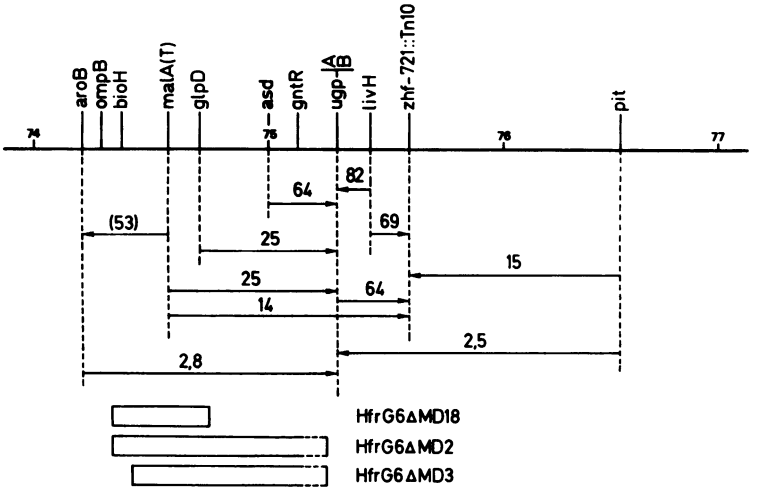

FIG. 2. Linkage of *ugpA704*::Tn*10* to other loci in the *aroB-pit* region. Positions of the indicated markers in the 74- to 77-min region of the *E. coli* chromosome are redrawn from Bachmann and Low (4). The numbers below are cotransduction frequencies, with the arrowhead designating the selected marker. The cotransduction frequency in parentheses between *malA* and *aroB* is from Anderson and Oxender (1). The bars indicate deletions covering *malA* and *glpD* and extending into the *asd* and *gntR* region (31).

TABLE 3. Transductional analysis of the position of *ugpA704*::Tn*10* (donor, SH1200 *ugpA704*::Tn*10* *malA*$^+$ *livH*$^+$; recipient, AE4107 *malA1* *livH*::Mu)

Cross	Selected marker	Recombinant class	% of total
A	Tc^r	*mal*$^+$ *livH*$^{+a}$	24.8 (124/500)
		mal$^+$ *livH*	8.8 (44/500)
		mal livH$^+$	57.0 (285/500)
		mal livH	9.4 (47/500)
B	MalA$^+$	*livH*$^+$ Tc^r	7.7 (46/600)
		livH$^+$ Tc^s	0.3 (2/600)
		livH Tc^s	90.2 (541/600)
		livH Tc^r	1.8 (11/600)

[a] *livH*$^+$ scored as ability to use 100 μg of D-leucine per ml as a L-leucine source.

(*ugpA704*::Tn*10*) was subjected to a selection for tetracycline susceptibility, mutants could be isolated with high frequency that had lost the G3P-binding protein but were still derepressed for the *pho* regulon. Since the appearance of tetracycline susceptibility is often accompanied by the formation of deletions or inversions into the neighboring genes, the structural gene for the binding protein must be closely linked to *ugpA704*::Tn*10*. This conclusion was corroborated by transducing strain SH306 (*ugpB703*::Mud (Amp^r *lac*) with a P1 lysate grown on SH1200 (*ugpA704*::Tn*10*). Of 37 Tc^r transductants, 36 were ampicillin susceptible (Amp^s) and synthesized the binding protein. Only one was ampicillin resistant and lacked the binding protein. The periplasmic proteins of strains SH306 and SH1200 as well as a Tc^r Amp^s transductant, SH1238, were analyzed by immunodiffusion against purified anti-G3P-binding protein antibodies (Fig. 3). The presence or absence of G3P-binding protein in the different strains was evident. The same result was obtained by analyzing

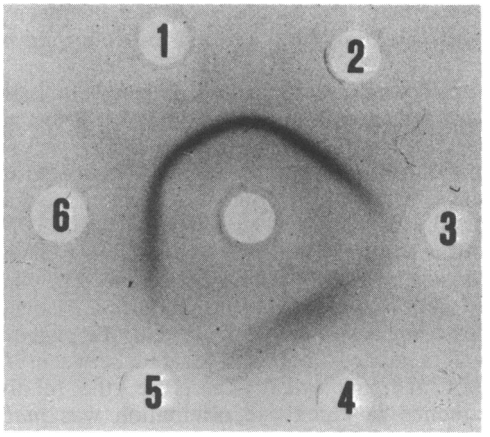

FIG. 3. Presence of G3P-binding protein in different mutants. Periplasmic proteins of different *ugp* mutants were isolated by the spheroplast formation technique, and Ouchterlony double-immunodiffusion assays were carried out. The center well contained purified rabbit antibodies against G3P-binding protein. The outer wells contained periplasmic proteins obtained from *ugpA704*::Tn*10* strain SH1200 (1), *ugp*$^+$ strain SH101 (2), *ugpB703*::Mud (Amp^r *lac*) strain SH306 (5), and *ugpA704*::Tn*10* transductants of SH306 (SH1238) (4 and 6). Well 3 contained no protein.

periplasmic proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (not shown). From the above-described transduction, it is clear that the insertion of Mud (Amp^r *lac*) into *ugpB* that resulted in the absence of G3P-binding protein was closely linked to *ugpA*. *ugpB* could be the structural gene for the G3P-binding protein. However, since Mu insertions are strongly polar, it may also be proximal to it, on the same operon. To determine the relative order of *ugpA*, *ugpB*, and *malT*, the cross shown in Table

TABLE 4. Transductional analysis of the position of *zhf-721*::Tn*10* (selection for Tc^r)

Cross	Strains (relevant genotype)		Recombinant class	% of total
	Donor	Recipient		
1	SH43 *zhf-721*::Tn*10*	AE4107 *malA1* *livH*::Mu	*livH*$^+$ *mal*$^+$	7.5 (15/200)
			livH$^+$ *mal*	61.0 (122/200)
			livH mal	30.5 (61/200)
			livH mal$^+$	1.0 (2/200)
2	SH43 *zhf-721*::Tn*10*	SH606 *malT* *ugpB703*::Mud (Amp^r *lac*)	*mal*$^+$ Amp^s	19.0 (19/100)
			mal$^+$ Amp^r	1.0 (1/100)
			mal Amp^s	38.0 (38/100)
			mal Amp^r	42.0 (42/100)
3	SH44 *zhf-721*::Tn*10*	SH606 *malT* *ugpB703*::Mud (Amp^r *lac*)	*mal*$^+$ Amp^s	17.0 (17/100)
			mal$^+$ Amp^r	1.0 (1/100)
			mal Amp^s	55.0 (55/100)
			mal Amp^r	27.0 (27/100)
4	SH43 *zhf-721*::Tn*10* *ugp-702*	SH123 *livH*::Mu	*livH*$^+$ *ugp*$^+$	12.5 (25/200)
			livH$^+$ *ugp*	58.0 (116/200)
			livH ugp	5.5 (11/200)
			livH ugp$^+$	24.0 (48/200)

5 was done. As can be seen, the presence of *ugpB703*::Mud (Amp^r *lac*) on the donor did not decrease the cotransduction frequency of *ug-pA704*::Tn*10* with *malT* (compare with Tables 2 and 3). Since phage Mu comprises close to 1 min in DNA length, *ugpB703*::Mud (Amp^r *lac*) has to be located outside the markers *malT* and *ug-pA704*::Tn*10*. A control experiment to validate this conclusion is also shown in Table 5. The cotransduction frequency of *zhf-721*::Tn*10* with *malT* of 10 to 18% (Table 2) became zero when the same cross was performed with a P1 lysate grown on a strain that carried a Mu phage in *livH*, a marker that was located between *zhf-721*::Tn*10* and *malT*. Therefore, the relative sequence in clockwise orientation was *malT*, *ugpA*, *ugpB*, *livH*.

DISCUSSION

The *ugp* genes are part of the *pho* regulon, but they are not linked to any of the known *pho*-regulated operons. They are located at 75.3 min on the *E. coli* linkage map, far away from *phoA* (87 min) (24), *phoE* (5.8 min) (28), and *phoS* (83.1 min) (2). There are at least two *ugp* genes. One, *ugpA*, is defined by a Tn*10* insertion that results in the loss of G3P transport activity but still allows the synthesis of the G3P-binding protein. Since Tn*10* insertion exhibits strong polar effects toward distal genes, *ugpA* is located either distal to the structural gene of the G3P-binding protein on a multicistronic operon or on a separate but closely linked operon. The second gene, *ugpB*, is defined by the insertion of phage Mud (Amp^r *lac*), which also results in the loss of G3P transport activity but, in addition, prevents the synthesis of the G3P-binding protein. Therefore, the insertion is either in the structural gene of the G3P-binding protein or in a gene that is proximal to it. Both genes *ugpA* and *ugpB* are closely linked, and their relative order could be determined in clockwise direction as *malA(T)*, *ugpA*, *ugpB*, *livH*.

So far, a positive selection for *ugp*$^+$ is not available, since *ugp*$^+$ *glpT* strains do not grow on G3P. However, as shown previously (25), G3P transported via the *ugp* transport system, in cells that grow on another carbon source, is able to satisfy the growth requirement for G3P in a mutant carrying a defective G3P-acyltransferase (10). This technique can now be used to map a number of *ugp* mutations by complementation analysis and so determine the number and order of the *ugp* genes.

TABLE 5. Transductional analysis of the relative sequence of *ugpA* and *ugpB* to *malT* (selection for Tc^r)

Strains (relevent genotype)		Recombinant class	% of total
Donor	Recipient		
SH1239 *ugpA704*::Tn*10* *ugpB703*::Mud (Amp^r *lac*)	SH606 *malT*	*mal*$^+$	31 (31/100)
	ugpB703::Mud (Amp^r *lac*)	*mal*	69 (69/100)
SH46 *zhf-721*::Tn*10* *livH*::Mu *malAl*	SH47 *livH*::Mu	*mal*$^+$	100 (100/100)
		mal	0 (0/100)

ACKNOWLEDGMENTS

We thank B. Bachmann, M. Casadaban, E. C. C. Lin, D. Ludtke, D. Oxender, and B. Wanner for supplying mutants and T. Larson for stimulating discussions.

This work was supported through grants from the Deutsche Forschungsgemeinschaft (SFB 138), the Fond der Chemischem Industrie, and NATO.

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