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sn-Glycerol-3-Phosphate Auxotrophy of *plsB* Strains of *Escherichia coli*: Evidence that a Second Mutation, *plsX*, Is Required

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***sn*-Glycerol-3-phosphate auxotrophs defective in phospholipid synthesis contain a K_m-defective *sn*-glycerol-3-phosphate acyltransferase. Detailed genetic analysis revealed that two mutations were required for the auxotrophic phenotype. One mutation, in the previously described *plsB* locus (*sn*-glycerol-3-phosphate acyltransferase structural gene), mapped near min 92 on the *Escherichia coli* linkage map. Isolation of Tn*10* insertions cotransducible with the auxotrophy in phage P1 crosses revealed that a second mutation was required with *plsB26* to confer the *sn*-glycerol-3-phosphate auxotrophic phenotype. This second locus, *plsX*, mapped between *pyrC* and *purB* near min 24 on the *E. coli* linkage map. Tn*10* insertions near *plsX* allowed detailed mapping of the genetic loci in this region. A clockwise gene order *putA pyrC flbA flaL flaT plsX fabD ptsG thiK purB* was inferred from results of two- and three-factor crosses. Strains harboring the four possible configurations of the mutant and wild-type *plsB* and *plsX* loci were constructed. Isogenic *plsB*$^+$ *plsX*$^+$, *plsB*$^+$ *plsX50*, and *plsB26 plsX*$^+$ strains grew equally well on glucose minimal medium without *sn*-glycerol-3-phosphate. In addition, *plsX* or *plsX*$^+$ had no apparent effect on *sn*-glycerol-3-phosphate acyltransferase activity measured in membrane preparations. The molecular basis for the *plsX* requirement for conferral of *sn*-glycerol-3-phosphate auxotrophy in these strains remains to be established.**

The initial reaction of membrane phospholipid synthesis in *Escherichia coli* is catalyzed by the membrane-bound enzyme, *sn*-glycerol-3-phosphate (glycerol-P) acyltransferase. Mutants defective in this enzyme have been isolated as glycerol-P auxotrophs (4). The auxotrophic phenotype of these mutants is due to a defective glycerol-P acyltransferase which has (among other distinguishing properties [5]) an elevated K_m for glycerol-P. The structural gene (*plsB*) for the glycerol-P acyltransferase is located near min 92 on the linkage map of *E. coli* (2, 28). Glycerol-P auxotrophic strains harboring the altered glycerol-P acyltransferase have been useful for studying the effects of cessation of phospholipid synthesis upon macromolecular synthesis, assembly of membrane proteins, protein secretion, and bacteriophage infection (36). In addition, the auxotrophic phenotype has permitted the selection of clones harboring the wild-type *plsB* locus (28); these clones have facilitated identification, overproduction, and purification of the glycerol-P acyltransferase, and sequencing of the *plsB* gene (19, 26, 27). The glycerol-P auxotrophy associated with *plsB* strains has enabled genetic selection for the *ugp*-encoded active transport system for glycerol-P (39, 40).

Biochemical and genetic studies of the glycerol-P acyltransferase would be facilitated if the glycerol-P auxotrophy of *plsB* strains could be transferred into other genetic backgrounds. When this proved to be impossible by P1 transduction into various recipients (including strain 8, the parent of the glycerol-P auxotrophs), the search for a second mutation required for the auxotrophy was undertaken. Studies on the identification and mapping of this second locus, *plsX*, are reported.

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MATERIALS AND METHODS

Bacterial strains and growth media. The bacterial strains utilized or constructed are listed in Table 1. Preparation of phage lysates and transductions with P1*vir* were carried out as recommended by Miller (34).

For minimal media, the A and B salts of Clark and Maaløe (11) were supplemented with 2 μg of thiamine per ml and 0.2 to 0.4% of the various carbon sources. Minimal media also contained 20 mM sodium citrate for the selection or scoring of transductants.

Tet^r transductants were selected on Difco nutrient agar (1.5%) containing 5 μg of tetracycline hydrochloride per ml, 20 mM sodium citrate, 0.1% glucose, and 0.01% glycerol-P. Inheritance of nearby markers was then scored on the following media containing 20 mM sodium citrate and 20 μg of tetracycline HCl per ml: *putA*, tetrazolium-proline indicator medium (8); *pyrC*, minimal glucose medium without uracil; *flbA*, *flaL*, or *flaT*, tryptone swarm agar (35); *fabD*, minimal glycerol or glucose medium at 42°C; *ptsG*, minimal medium containing 1% glucose, 0.2% lactose, and 20 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside per ml; *thiK*, minimal glucose medium containing 20 nM thiamine HCl; *plsB* or *plsX*, minimal glucose or maltose medium; *dgkA*, Difco antibiotic medium 2 or Luria broth with no sodium chloride added (sodium citrate was omitted from these media); and *malE*, minimal maltose medium.

Kan^r transductants were selected and scored on similar media with kanamycin sulfate at 50 μg per ml.

Tetracycline-sensitive (Tet^s) derivatives of strains harboring Tn*10* insertions were isolated with media containing fusaric acid (7, 33).

Preparation of cell envelope fractions and assay of glycerol-P acyltransferase. Cells grown overnight at 37°C in 250 ml of LB medium (34) supplemented with 0.1% glucose and 0.01% glycerol-P were used in the following steps which were carried out at 0 to 4°C. Pelleted cells were washed with 50 ml of 10 mM Tris-hydrochloride (pH 8)–5 mM MgCl and then disrupted by passage through a French pressure cell.

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

Strain	Genotype	Source or reference[a]
8	HfrC *phoA8 glpD3 glpR2 relA1 spoT1 pit-10 fhuA22 ompF627 fadL701* (λ)	21
BB2636	8 *glpK*^{i} *plsB26 plsX50*	4
G19	F^{-} *putA purE gltA his lac rpsL*	CGSC 5757 (12)
X7014a	F^{-} *pyrC46 purB51 rpsL125 thi-1 lacZ malA1 mtl-2 xyl-7*	CGSC 5358 (42)
CS101-AP2U1	Hfr *pyrC44 metB1 relA1 fhuA22* T2^{r}	CGSC 5151
MA1008(30SOU6)	HfrH *pyrC46 thi-1 relA1 lacZ43*	CGSC 5153 (3)
YK2861	F^{-} *flbA2861 galK2 uvrA157 rpsL200* (λ *ind*$^{-}$)	24
YK4118	F^{-} *flaL4118 araD139* Δ(*argF-lac*)*U169 rpsL thi pyrC46 gyrA thyA his*	24
YK4176	F^{-} *flaT4176 araD139* Δ(*argF-lac*)*U169 rpsL thi pyrC46 gyrA thyA his*	24
L48	F^{-} *fabD89 gltA5 rpsL20 thi-1 lct-1 ara-14 lacY1 galK2 xyl-5 mtl-1 tsx-57 tfr-5 supE44* (λ)	CGSC 5638 (42)
LA-12	F^{+} *ptsG21 thi-1 relA1 spoT1*	CGSC 5084 (30)
NI510	F^{-} *thiD1 thiK1 argG6 metB1 his-1 leu-6 trp-31 lacY1 rpsL104 gal-6*	23
PC0254	F^{-} *purB51 trp-45 his-68 tyrA2 thi-1 lacY1 malA1 gal-6 xyl-7 mtl-2 rpsL125 fhuA2*	CGSC 5038
CY288	F^{-} or F^{+} *fabF200 fabB15 zcf-229*::Tn*10 gyrA220 rpsL146 relA1? pit-10? spoT1? fhuA22*? T2^{r}?	CGSC 6356 (16)
RZ6	F^{-} *dgkA6 thr-1 leu-6 his-4 rpsL136 fhuA*	37
pop1740	HfrG6 *his* Δ*malB112*	M. Hofnung
DL114	pop1740 *zjb-750*::Tn*10 malB*$^{+}$	P1 · Tn*10* pool→pop1740
MC4100	F^{-} *araD139* Δ(*argF-lac*)*U169 rpsL150 deoC1 relA1 thiA ptsF25 flbB5301*	10
TS100	MC4100 *glpR2*	1
TST3	MC4100 *malT*::Tn*10*	(T. J. Silhavy)
HS3018	MC4100 *malT*^{c}-1 Δ*malE444*	41
TL48	MC4100 *glpD3 glpR2*	P1 · BB2636→TST3
TL84	BB2636 *zjb-750*::Tn*10*	P1 · DL114→BB2636
TL85	BB2636 *gyrA*	P1 · DL291→BB2636 (32)
TL112	TL85 *zce-726*::Tn*10 plsX*$^{+}$	P1 · Tn*10 pool→TL85*
TL125	TL85 *zce-727*::Tn*10 plsX*$^{+}$	P1 · Tn*10* pool→TL85
TL126	TL85 *zce-728*::Tn*10 plsX*$^{+}$	P1 · Tn*10* pool→TL85
TL129	TL85 *zjb-729*::Tn*10 plsB*$^{+}$	P1 · Tn*10* pool→TL85
TL137	BB2636 *zjb-729*::Tn*10*	P1 · TL129→BB2636
TL140	BB2636 *zce-727*::Tn*10*	P1 · TL125→BB2636
TL145,146[b]	TL48 *plsB26 zjb-729*::Tn*10*	P1 · TL137→TL48
TL147,148	TL145,146 Tet^{s}	Fusaric acid selection
TL150	TL148 *zce-727*::Tn*10 plsX50*	P1 · TL140→TL148
TL153,154	TL48 *zce-727*::Tn*10 plsX50*	P1 · TL140→TL48
TLK155,156	TL153,154 Tet^{s}	Fusaric acid selection
TL157	TL48 *zce-727*::Tn*10 plsX50*	P1 · TL140→TL48
TL158	TL157 *pyrC* Tet^{s}	Fusaric acid selection
TL212	HS3018 *zce-726*::Tn*10*	P1 · TL112→HS3018
TL225	HS3018 *zce-727*::Tn*10*	P1 · TL125→HS3018
TL226	HS3018 *zce-728*::Tn*10*	P1 · TL126→HS3018
TL229	HS3018 *zjb-729*::Tn*10 malE*$^{+}$	P1 · TL129→HS3018
TL236,237	MC4100 *plsX50*	(i) P1 · TL140→MC4100 (ii) Tet^{s} selection
TL238,239	TS100 *plsX50*	(i) P1 · TL140→TS100 (ii) Tet^{s} selection
TL256,257	MC4100 *zjb-729*::Tn*10 plsB26 plsX50*	P1 · TL137→TL236,237
TL258,259	MC4100 *zjb-729*::Tn*10 glpR2 plsB26 plsX50*	P1 · TL137→TL238,239
TL391,392	MC4100 *zjb-750*::Tn*10 plsB26*	P1 · TL84→MC4100
TL393,394	MC4100 *zjb-750*::Tn*10 glpR2 plsB26*	P1 · TL84→TS100
TL247,248	LA-12 *zce-727*::Tn*10*	P1 · TL225→LA-12
TL266	L48 Lac^{+}	P1 · TL125→L48
TL270	L48 *zce-727*::Tn*10*	P1 · TL225→L48
TL271,272	MC4100 *zcf-229*::Tn*10* (*fabF200*?)	P1 · CY288→MC4100
TL329	LA-12 *zcf-229*::Tn*10* (*fabF200*?)	P1 · TL271→LA-12
HS3020	MC4100 *malE*::Tn*5*-7 *malT*^{c}-1 Φ(*malP-lac*)	41
TL321	BB2636 *malE*::Tn*5*-7 *plsB*$^{+}$	P1 · HS3020→BB2636
TL322	BB2636 *malE*::Tn*5*-7	P1 · HS3020→BB2636
TL323	TL321 *zce-727*::Tn*10 plsX*$^{+}$	P1 · TL225→TL321
TL324	TL321 *zce-727*::Tn*10*	P1 · TL157→TL321
TL325	TL322 *zce-727*::Tn*10 plsX*$^{+}$	P1 · TL225→TL322
TL326	TL322 *zce-727*::Tn*10*	P1 · TL157→TL322

[a] CGSC, Coli Genetic Stock Center, Yale University, New Haven, Conn.

[b] The listing of two consecutive strain numbers indicates that two independent isolates were saved from the indicated derivation.

TABLE 2. Transductional mapping of Tn*10* insertions near the *plsX* locus

Recipient strain	%[a] in following P1 donor strain			
	TL212 *zce-726*::Tn*10*	TL225 *zce-727*::Tn*10*	TL226 *zce-728*::Tn*10*	TL271 *zcf-229*::Tn*10*[b]
BB2636 *plsX50*	86.5 (173/200)	98 (49/50)	97 (194/200)	21.3 (64/300)
G19 *putA*	0.5 (1/200)	2.9 (6/210)	2.5 (5/200)	ND
X7014a *pyrC46*	8.5 (17/200)	11.5 (23/200)	11 (22/200)	2 (2/100)
YK2861 *flbA2861*	13.6 (14/103)	36.3 (37/102)	33 (33/100)	ND
YK4118 *flaL4118*	49 (49/100)	57 (57/100)	55 (55/100)	ND
YK4176 *flaT4176*	66.7 (68/102)	82.6 (81/98)	79.2 (80/101)	ND
L48 *fabD89*	88.7 (118/133)	85.6 (154/180)	90.8 (69/76)	24.5 (49/200)
LA-12 *ptsG21*	85 (170/200)	61 (183/300)	63 (126/200)	27 (54/200)
NI510 *thiK*	64.7 (194/300)	58.6 (178/304)	63.2 (192/304)	35.5 (71/200)

[a] Tet^r was selected in each cross. The cotransduction frequencies shown are the percentages of the Tet^r transductants which contained the unselected marker.
[b] ND, not done.

After DNase treatment, unbroken cells were removed by centrifugation (10,000 × *g* for 10 min). The particulate fraction was then obtained by centrifugation at 200,000 × *g* for 1 h. The resulting pellet, homogenized in 2 ml of 50 mM Tris-hydrochloride (pH 8), was used as the source of glycerol-P acyltransferase. This activity was measured at 25°C as described previously (28), with 40 μM palmitoyl coenzyme A as the acyl donor. Activity was proportional with time and the amount of protein used. One unit of enzyme activity is defined as the amount of enzyme required to convert 1 nmol of ^{3}H-glycerol-P into chloroform-soluble material per min.

Protein concentrations were estimated by the method of Lowry et al. (31), with bovine serum albumin as the standard.

RESULTS

The glycerol-P auxotrophy associated with *plsB26* is not cotransduced with *zjb-750*::Tn*10*. To facilitate the construction of *plsB26* strains by phage P1 transduction, a strain containing the transposon Tn*10* next to *plsB26* was constructed. P1 transduction with strain DL114 (*zjb-750*::Tn*10*) as the donor and strain BB2636 (*plsB26*) as the recipient was performed, selecting Tet^r and scoring the glycerol-P auxotrophy. This Tn*10* was 94% cotransducible with *plsB*. By using a lysate of one of the *plsB26* Tet^r colonies (TL84) from the above cross as the P1 donor, the glycerol-P auxotrophic phenotype previously associated with *plsB26* was not cotransduced with the Tn*10* insertion into a variety of recipient strains, including strain 8, the parent of strain BB2636. Therefore, the hypothesis that two (or more) mutations are required for the glycerol-P auxotrophic phenotype of BB2636 was considered.

Identification of a second mutation *plsX*. To test this hypothesis, independent Tn*10* insertions from wild-type cells were sought which would correct the auxotrophy by P1-mediated cotransduction. This was done by transduction with a P1 lysate prepared on a collection of strain MC4100 derivatives (32), harboring random Tn*10* insertions (Tn*10* pool, Table 1) with strain TL85 (*plsB26*) as the recipient. Tet^r transductants obtained on rich medium were replica plated onto minimal glucose medium containing tetracycline. Twenty-two transductants no longer auxotrophic for glycerol-P were thus obtained. To determine whether the Tn*10* insertions in these transductants were located near *plsB*, they were used as P1 donors in crosses with strains HS3018 (Δ*malE444*), RZ6 (*dgkA6*), and BB2636 (*plsB26*) as recipients, with selection for Tet^r. These crosses with strain BB2636 as the recipient verified the cotransducibility of each of the Tn*10*'s with the glycerol-P auxotrophy. The other crosses indicated that 19 of the Tn*10* insertions were cotransducible with Δ*malE444*, *dgkA6* or both and thus mapped in the *plsB* region (e.g., TL129 [*zjb-729*::Tn*10*], Table 1). However, three Tn*10* insertions were identified which transduced strain TL85 to glycerol-P prototrophy but were not cotransducible with either *dgkA6* or Δ*malE444* (strains TL112, TL125, and TL126, Table 1). Because *dgkA* is located directly adjacent to *plsB* (27), these Tn*10* insertions must be located elsewhere on the *E. coli* chromosome, presumably near a second gene which must be mutated along with *plsB* to confer the glycerol-P auxotrophy. This second locus was named *plsX*.

Mapping of *plsX*. To map the position of *plsX* on the *E. coli* chromosome, the position of the Tn*10* insertion in strain TL125 was determined. This transposon and *plsX* are more than 95% cotransducible (Table 2). Time-of-entry experiments with HfrC strains TL125 and TL129 as donors and strain MC4100 as the recipient (selection for Tet^r) indicated that *plsX* was far removed from *plsB*. The Tn*10* near *plsX* required at least 50 min longer for entry compared with the Tn*10* near *plsB*.

Further mapping of the Tn*10* near *plsX* was facilitated when it was noted that deletion of this transposon gave rise to a uracil auxotrophy, correctable by cotransduction with the same Tn*10*. This uracil auxotroph (strain TL158) was used to isolate complementing λgt7 phage from a library of *E. coli* chromosomal *Eco*RI fragments (40). These phage complemented defined *pyrC* mutations. Thus *plsX* was located near *pyrC*, at min 24 on the *E. coli* linkage map.

To determine the position of *plsX* with respect to *pyrC* and other genetic loci in this region, two- and three-factor transductional analyses were performed. In two-factor crosses (Table 2), strains harboring the three different Tn*10* insertions near *plsX*$^+$ served as P1 donors for strains defective in various neighboring loci. Tet^r was selected in each cross, and the neighboring locus was scored as the unselected marker. These crosses suggested an arrangement of genetic loci (Fig. 1). Three-factor crosses (Table 3) were also consistent with the order shown in Fig. 1. Cross 1 (Table 3) demonstrated that *ptsG* is not between *pyrC* and *zce-727*::Tn*10* and thus must be clockwise from *plsX*. The *fabD* locus was located clockwise from *zce-727*::Tn*10* (and hence clockwise from *plsX*) (cross 2, Table 3). Because *ptsG* was not located between *zce-727*::Tn*10* and *fabD* (cross 3, Table 3) but was between *zcf-229*::Tn*10* and *fabD* (cross 4), the clockwise order *pyrC zce-727*::Tn*10 fabD ptsG* was indi-

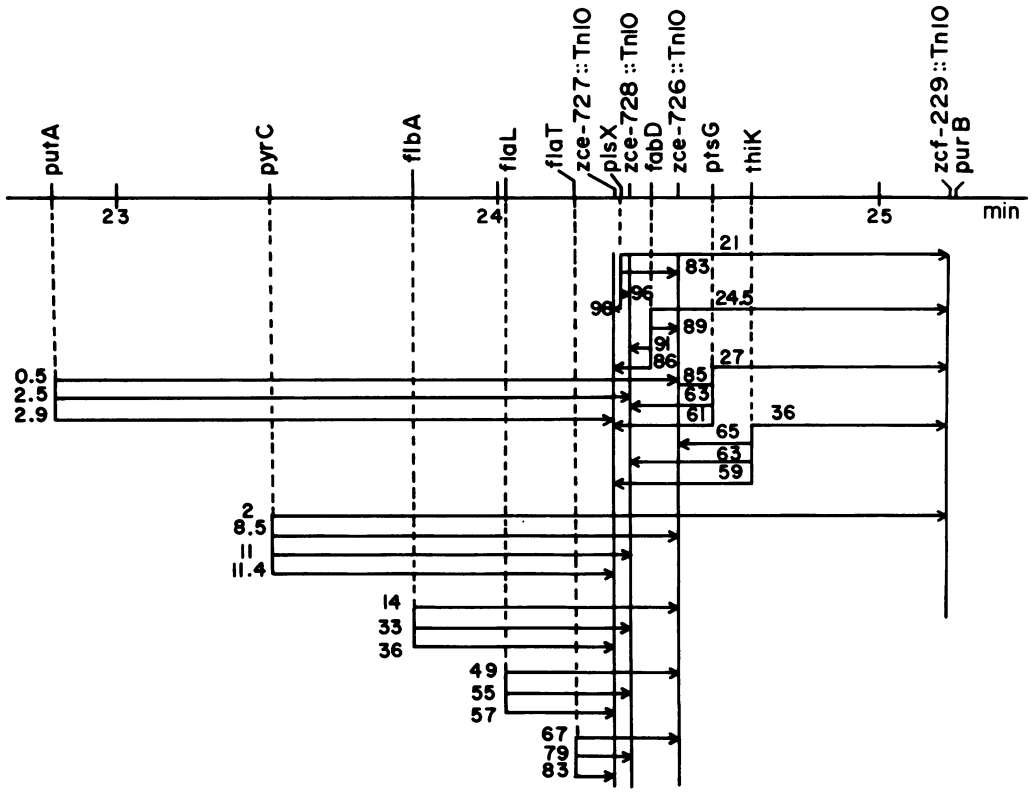

FIG. 1. Map positions of genetic loci and Tn*10* insertions in the *putA-purB* region of the *E. coli* chromosome. The arrows point to the Tn*10* selected in each cross, with the numbers indicating the observed cotransduction frequencies, in percent (derived from data in Table 2 and other data not shown). The *putA* and *pyrC* loci have been drawn in the positions indicated by Bachmann (2). The other positions were estimated from the observed cotransduction frequencies by the formula of Wu (44). For these distance calculations, the effective length of the P1 transducing fragment was reduced to 1.8 min of DNA (instead of 2 min) because of the necessity to package the Tn*10* in each case. The distance between *pyrC* and *zcf-229*::Tn*10* (*purB*) was ca. 1.7 min, using a variety of the indicated frequencies.

cated (Fig. 1). The results of cross 5 (Table 3) were also consistent with this order. The two-factor crosses (Table 2) were sufficient to place *thiK* and the flagellar genes as shown in Fig. 1; such placement agrees with previous mapping experiments placing *thiK* clockwise from *fabD* and *ptsG* (23) and placing the flagellar genes between *pyrC* and *ptsG* (25).

Anomolous results were obtained when the Tn*10* insertions were mapped with respect to *purB*. The P1 donors TL225 (*zce-727*::Tn*10*) or TL271 (*zcf-229*::Tn*10*) did not transduce either strain X7014a (*purB51*) or PC0254 (*purB51*) to Pur^+ when Tet^r was selected. Anomolous results were reported previously when selecting $ThiK^+$ and scoring *purB* (23) or when selecting $PurB^+$ from P1 donors containing *ptsG* or *fabD* and scoring *pyrC* (41). Because of the difficulties, *zcf-229*::Tn*10* served to mark the position of *purB*. This insertion is highly cotransducible with *purB* (99%; 16) and was arbitrarily placed to the left of *purB* (Fig. 1). The aberrant linkages between the Tn*10* insertions and *purB*

TABLE 3. Three-factor transductional analysis of genetic loci in the *plsX* region

Cross	Relevant genotype		Selected marker	Unselected marker	% with unselected marker
	Donor	Recipient			
1	TL247 *ptsG21 zce-727*::Tn*10*	CS101-AP2U1 *pyrC44*	Tet^r $pyrC^{+a}$	*ptsG21*	41 (80/196)
2	TL270 *fabD89 zce-727*::Tn*10*	MA1008 *pyrC46*	Tet^r $pyrC^{+b}$	*fabD89*	68 (68/100)
3	TL247 *ptsG21 zce-727*::Tn*10*	TL266 *fabD89*	Tet^r $fabD^{+c}$	*ptsG21*	66 (42/64)
4	TL329 *ptsG21 zcf-229*::Tn*10*	TL266 *fabD89*	Tet^r $fabD^{+c}$	*ptsG21*	93 (25/27)
5	TL270 *fabD89 zce-727*::Tn*10*	MA1008 *pyrC46*	Tet^{rd}	$pyrC^+$ *fabD89*	12 (34/293)
				$pyrC^+$ $fabD^+$	9 (27/293)
				pyrC46 fabD89	67 (196/293)
				pyrC46 $fabD^+$	12 (36/293)

[a] Selected on minimal medium containing 1% glucose, 0.2% lactose, 20 mM sodium citrate, 0.05% Casamino Acids, 50 μg of methionine per ml, 5 mM potassium glutamate, 5 μg of tetracycline HCl per ml, and 20 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside per ml. Coinheritance of *ptsG21* was directly observable on the selective plates.

[b] Selected at 30°C on glucose minimal medium containing 20 mM sodium citrate and 5 μg of tetracycline HCl per ml.

[c] Selected on the same medium as for cross 1 but at 42°C.

[d] Selected at 30°C on nutrient agar containing 5 μg of tetracycline HCl per ml, 20 mM sodium citrate, and 0.1% glucose. After 2 days, *pyrC* and *fabD* were scored as described in the text.

might be explained by the presence of the genetic element *e*14 (20), which behaves as a defective prophage (43). It is present in some, but not all, strains and has an attachment site near *purB* (20, 43).

The position of *plsX* with respect to the three nearby Tn*10* insertions was not determined. The results of two-factor crosses (Table 2) suggested that *zce-726*::Tn*10* is between *fabD* and *ptsG* as shown in Fig. 1, but this was not proven.

Introduction of *plsX*$^+$ by transduction into strains BB13 and BB26 (the parent of strain BB2636 [4]) resulted in the correction of the glycerol-P auxotrophy of these strains. *plsX*$^+$ did not correct the glycerol-P auxotrophy of strain BB2014 (*gpsA20*) which is missing the biosynthetic glycerol-P dehydrogenase (4).

Transfer of glycerol-P auxotrophy into other genetic backgrounds. If *plsB26* and *plsX50* are the only defects required for conferral of the glycerol-P auxotrophy on strain BB2636, then it should be possible to transfer the auxotrophy into other strains with two sequential P1 transductions. P1 lysates of strains TL137 (*plsB26 plsX50 zjb-729*::Tn*10*) and TL140 (*plsB26 plsX50 zce-727*::Tn*10*) were prepared to determine whether this is the case. Strain TL48, a *glpD3 glpR2* derivative of strain MC4100, was chosen as the recipient. These *glp* alleles were chosen to prevent catabolism (*glpD*) of the glycerol-P supplement and to provide constitutive (*glpR*) active transport of glycerol-P (29). All Tet^r transductants resulting from the cross TL48 × TL140 grew on glucose minimal medium without glycerol-P supplementation (strains TL153 and 154, Table 1). All Tet^r colonies resulting from the transductional cross TL48 × TL137 also grew without glycerol-P supplementation (strains TL145 and 146, Table 1).

Tet^s derivatives of the above transductants were selected to facilitate the introduction of the second mutation (strains TL147 and 148, and TL155 and 156, Table 1). The appropriate P1 donor (strain TL137 or TL140) was used to introduce the second defect by selecting for the neighboring Tn*10*. In both cases transductants auxotrophic for glycerol-P were obtained at the expected frequencies (e.g., strain TL150, Table 1).

Additional experiments were carried out with several other recipients of differing genetic backgrounds harboring *glpD3* and *glpR2*, including strain 8. In all instances, it was possible to transfer the glycerol-P auxotrophy with two sequential P1 transductions, as described above (data not shown). These results demonstrate that both *plsB26* and *plsX50* are necessary and sufficient for conferral of the glycerol-P auxotrophy.

Effect of *plsX* and *glpR* on growth and glycerol-P supplementation of *glpD*$^+$ *plsB26* strains. Strains were constructed to assess the necessity of the *glpD*, *glpR*, and *plsX* mutant alleles for the glycerol-P auxotrophy and for glycerol-P supplementation of strains harboring *plsB26*. For these experiments, *glpD*$^+$ strains with the four possible configurations of the *plsX* and *glpR* wild-type and mutant alleles (TL236,237 [*glpR*$^+$ *plsX50*], TL238,239 [*glpR2 plsX50*], MC4100 [*glpR*$^+$ *plsX*$^+$], and TS100 [*glpR2 plsX*$^+$] (Table 1) were used as recipients in P1 crosses where *plsB26* was introduced by cotransduction with a neighboring Tn*10*. The *plsB26* allele could be introduced when selection for Tet^r was carried out on nutrient agar containing 0.4% glycerol; Tet^r *plsB26* transductants were not obtained when selection was carried out on nutrient agar containing 0.1% glucose and 0.01% glycerol-P. Tet^r *plsB26* transductants were unable to grow on this glucose-nutrient agar and were thus distinguished from their Tet^r *plsB*$^+$ siblings. When the transductants were tested on minimal media, the results shown in Table 4 were obtained. The Tet^r *plsB*$^+$ transductants from all four crosses grew on all of these media (data not shown). Strains defective in *plsX* and *plsB* (TL256,257 and TL258,259) were unable to grow on glucose even with glycerol or glycerol-P supplementation, regardless of the state of *glpR*. These strains were able to grow on maltose with 0.1% glycerol but were unable to grow on maltose alone or maltose supplemented with 0.1% glycerol-P (data not shown). Strains that are *glpD3* but otherwise isogenic with respect to the alleles shown for TL258,259 (Table 4) exhibit the typical glycerol-P auxotrophic phenotype of *plsB* strains (e.g., strain TL150). Thus, constitutive (*glpR2*) active transport of glycerol-P together with a defective glycerol-P dehydrogenase (*glpD3*) is required for supplementation with glycerol-P during growth on glucose or maltose.

In the case of the *plsX*$^+$ strains (TL391 to 394, Table 4), the repressor for the members of the *glp* regulon (29) exerted an influence on the phenotypes. Here, *glpR*$^+$ allowed growth on glucose without supplementation. The *glpR2* derivatives did not grow on glucose or maltose but did grow when supplemented with either glycerol or glycerol-P. All of the strains (Table 4) grew on glycerol or glycerol-P minimal medium.

Effect of *plsX* on growth and glycerol-P acyltransferase activity. To determine whether the ability of *plsX*$^+$ *plsB26 glpD3* strains to grow without glycerol-P supplementation was due to a correction of the K_m defect of the acyltransferase, the enzyme activity was investigated in membranes from strains harboring the four possible configurations of *plsB* and *plsX* mutant and wild-type alleles. Neither the V_{max} nor the apparent K_m of the acyltransferase for glycerol-P was influenced by *plsX50* or *plsX*$^+$ (Table 5). The glycerol-P acyltransferase from strains harboring the *plsB26* allele had low V_{max}'s (0.7 to 0.9 U/mg) and high K_m's (1,000 μM), independent of the *plsX* allele. The glycerol-P acyltransferase activity from both *plsB*$^+$ strains had a high V_{max} (6.9 U/mg) and low K_m (120 μM). Similar relative specific activities were obtained when palmitoyl-acyl carrier protein was employed as the acyl donor (data not shown).

The thermolability of the glycerol-P acyltransferase activity was determined in membrane preparations from strains harboring the four possible configurations of *plsB* and *plsX* mutant and wild-type alleles (strains TL48, TL147, TL150, TL155, and TL323 to 326; data not shown). The activity from all strains harboring *plsB26* was thermolabile when compared with the activity from strains harboring *plsB*$^+$. The presence of *plsX*$^+$ did not influence thermolability significantly.

TABLE 4. Growth properties of *glpD*$^+$ *plsB26* strains harboring *plsX* and *glpR* wild-type or mutant alleles or both

Strain	Genotype	Growth properties in following growth medium[a]		
		Glucose	Glucose + glycerol-P or glycerol	Glycerol-P or glycerol
TL256,257	*plsB26 plsX50 glpR*$^+$	−	−	+
TL258,259	*plsB26 plsX50 glpR2*	−	−	+
TL391,392	*plsB26 plsX*$^+$ *glpR*$^+$	+	+	+
T1393,394	*plsB26 plsX*$^+$ *glpR2*	−	±	+

[a] Minimal medium contained 0.4% glucose or glycerol, or 0.2% glycerol-P. When added to the glucose medium, glycerol-P or glycerol was present at 0.1%. Strains were patched on the solid medium, and growth at 37°C was monitored after 20 h. −, No growth; +, strong growth; ±, weak growth.

TABLE 5. Glycerol-P acyltransferase activity in membranes from wild-type and mutant strains[a]

Strain	Genotype	V_{max} (U/mg)	K_m (μM) for glycerol-P
TL48	*plsB*$^+$ *plsX*$^+$	6.9	120
TL154	*plsB*$^+$ *plsX50*	6.9	120
TL145	*plsB26 plsX*$^+$	0.9	1,000
TL150	*plsB26 plsX50*	0.7	1,000

[a] Activities were determined with palmitoyl coenzyme A as the acyl donor.

Although the glycerol-P acyltransferase from *plsB26 plsX*$^+$ strains was of low specific activity, was thermolabile, and exhibited a high K_m for glycerol-P, such strains were capable of rapid growth on minimal glucose medium at 37°C. Strains TL48 (*plsB*$^+$ *plsX*$^+$), TL155 (*plsB*$^+$ *plsX50*), and TL147 (*plsB26 plsX*$^+$) grew equally well on glucuse minimal medium, with doubling times of ca. 50 min. The same results were obtained with isogenic strains derived from strain BB2636 (TL323 [*plsB*$^+$ *plsX*$^+$], TL324 [*plsB*$^+$ *plsX50*], and TL325 [*plsB26 plsX*$^+$]), with doubling times of 60 min. Supplementation of the *plsB26 plsX50* strains TL150 and TL326 with 0.02% glycerol-P resulted in growth with comparable doubling times.

DISCUSSION

Isolation of Tn*10* insertions near *plsX* enabled its identification and facilitated its mapping in the min 24 region of the *E. coli* linkage map. Mapping of *plsX* also clarified ambiguities concerning the positioning of certain loci in this region. With the exception of *rne*, *glnR*, and *fabF*, all of the genetic loci known to lie between *pyrC* and *purB* were mapped in the experiments reported here. *fabF* has been mapped just clockwise from *fabD* (16). The Tn*10* insertions near *plsX* could aid in more detailed mapping of *glnR* and *rne*. Bachmann (2) has pointed out that the distance between *pyrC* and *purB* has not been determined accurately. Before our work, a distance of either 1.4 or 2 min was estimated from results of different experiments (2). The cotransduction frequencies observed in the present study suggest a distance of 1.75 min, providing that *zcf-229*::Tn*10* accurately reflects the position of *purB*.

Previous mapping studies where selection was for glycerol-P prototrophy with *plsB* strains as recipients should be reevaluated, because the transductants arising from these crosses could have been either *plsB*$^+$ or *plsX*$^+$. This could explain the relatively low cotransduction frequency (66%) observed between *plsB* and *dgkA* (28), even though they are adjacent loci (27). The distance between *malB* and *plsB* also appears to be less than that estimated previously (28). A cotransduction frequency of 66% between *zjb-729*::Tn*10* and *plsB* was observed, which indicates a distance of ca. 0.23 min. This Tn*10* insertion is actually located in *malB*, between *malK* and *lamB* (9).

The Tn*10* insertions near *plsX* and *plsB* have made possible the transfer of the glycerol-P auxotrophy into other genetic backgrounds. For construction of such auxotrophs, selection for Tet^r encoded by a Tn*10* near *plsX50*, followed by selection for Kan^r encoded by *malE*::Tn*5* near *plsB26*, obviates the need to select a Tet^s intermediate after the first cross, as was done for construction of some strains described here. The ability to transfer the auxotrophy will facilitate further biochemical and genetic studies on glycerol-P acyltransferase and on the *ugp*-encoded active transport system for glycerol-P.

The mechanism by which *plsX* exerts its effect remains to be elucidated. It is possible that *plsX*$^+$ allows elevation of intracellular glycerol-P levels, thereby permitting the K_m-defective glycerol-P acyltransferase to function. That elevated glycerol-P levels allow the growth of *plsB26 plsX50* strains has been shown by isolation of glycerol-P prototrophic revertants having an altered biosynthetic (*gpsA*-encoded) glycerol-P dehydrogenase that is insensitive to feedback inhibition by glycerol-P (6, 13). The influence of *glpR* on phenotypes exhibited by *plsB26 plsX*$^+$ strains (Table 4) is consistent with the idea that elevated glycerol-P levels are required for growth of these strains. Glycerol-P levels may be lower in the *glpR2* strains when compared with that in the *glpR*$^+$ strains due to the presence of the catabolic (*glpD*-encoded) glycerol-P dehydrogenase in the constitutive strains. The phenotypic differences (Table 4) between strains TL256,257 (*plsB26 plsX50 glpR*$^+$) and TL391,392 (*plsB26 plsX*$^+$ *glpR*$^+$) could mean that *plsX* influences glycerol-P synthesis (the catabolic glycerol-P dehydrogenase would be effectively repressed in these strains during growth on glucose [29]). The biosynthetic glycerol-P dehydrogenase could be inhibited in *plsX* strains due to an accumulation of a metabolic intermediate. Along these lines, it may be interesting to note that acyl coenzymes A are potent inhibitors of the biosynthetic glycerol-P dehydrogenase when tested in vitro (14).

Another possibility is that *plsX* encodes a protein which interacts with or modulates glycerol-P acyltransferase activity. A functional interaction between adenylate kinase and glycerol-P acyltransferase has been proposed (15, 17), based in part on the observation that temperature-sensitive mutants defective in adenylate kinase have thermolabile glycerol-P acyltransferase activity (17). Because *adk* (encoding adenylate kinase) and *plsX* map at two distinct sites on the linkage map (2), *plsX* does not encode adenylate kinase. However, *plsX* could encode another protein required for interaction between adenylate kinase and glycerol-P acyltransferase, such as the adenylate kinase-associated protein (22) or one of the inner membrane-associated proteins precipitable by anti-adenylate kinase immunoglobulin G (18).

No effect of *plsX50* or *plsX*$^+$ on either wild-type or mutant glycerol-P acyltransferase activity was apparent in vitro. Most of the assays performed used palmitoyl coenzyme A as the acyl donor, although similar results were seen with palmitoyl-acyl carrier protein, the presumed physiological substrate (38). An effect of *plsX* on glycerol-P acyltransferase activity cannot be excluded since it may not have been detectable under the conditions used. Finally, the close proximity of the *plsX* locus to two genes involved in fatty acid biosynthesis (*fabD* and *fabF*) raises the intriguing possibility that *plsX* could be involved in fatty acid biosynthesis or its regulation. The ease with which *plsX* can now be genetically manipulated should facilitate studies on its function.

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