JOURNAL OF BACTERIOLOGY, Oct. 1974, p. 227-233
Vol. 120, No. 1

Mutants of *Escherichia coli* Defective in Membrane Phospholipid Synthesis: Mapping of *sn*-Glycerol 3-Phosphate Acyltransferase K_m Mutants

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Received for publication 19 July 1974

plsB mutants of *Escherichia coli* are *sn*-glycerol 3-phosphate auxotrophs which owe their requirement to a K_m defect in *sn*-glycerol 3-phosphate acyltransferase, the first enzyme in the phospholipid biosynthetic pathway. We have located the *plsB* gene at minute 69 of the *E. coli* genetic map, far removed from the gene defined by mutants with a temperature-sensitive *sn*-glycerol 3-phosphate acyltransferase. The *plsB* gene was cotransduced with the *dctA* locus, and the transduction data indicated that the clockwise gene order is *asd*, *plsB*, *dctA*, *xyl*. *plsB*$^-$ is recessive to *plsB*$^+$ and all acyltransferase K_m mutants tested lie very close to the *plsB* locus. Effective supplementation of *plsB* mutants was shown not to require a defective *glpD* gene.

Isolation and characterization of mutants of *Escherichia coli* defective in membrane synthesis have inaugurated a series of investigations into the structural and functional importance of phospholipids in biological membranes (8). *sn*-Glycerol-3-phosphate (G3P) is a precursor required for the synthesis of all the phospholipid species found in *E. coli* (8). Two classes of mutants requiring G3P (or glycerol) for growth have been isolated (2).

The first class of G3P auxotroph, called *gpsA* for glycerol phosphate synthesis, is defective in the structural gene for the biosynthetic G3P dehydrogenase (2, 5). This gene lies at minute 71 on the *E. coli* linkage map (5).

The second class of G3P auxotroph is not defective in G3P synthesis but owed its G3P requirement to defective utilization of G3P caused by an alteration in G3P acyltransferase, the first enzyme of phospholipid biosynthesis (2, 15). These strains have a membranous G3P acyltransferase activity with an apparent K_m for G3P that is 10-fold higher than that of the wild-type strain (2). G3P acyltransferase mutants with a temperature-sensitive (ts) enzyme activity have been mapped at minute 13 of the *E. coli* map (6). However, these two types of G3P acyltransferase mutant differ markedly in their behavior under nonpermissive conditions. The ts mutants cease growth and macromolecule synthesis abruptly when phospholipid synthesis is halted by temperature shift. In contrast, when phospholipid synthesis is halted by starvation of a K_m mutant for G3P growth and macromolecule synthesis continued for about one generation after starvation. Therefore, two types of mutations affecting the same enzyme activity have very different properties.

We undertook the present investigation to further characterize the G3P acyltransferase K_m mutants and to attempt to reconcile the differences between the phenotypes of the ts and the K_m types of G3P acyltransferase mutants. We show that the gene (*plsB*) defined by the G3P acyltransferase mutants is located at minute 69 of the linkage map, a site far removed from the gene (*plsA*) defined by the ts G3P acyltransferase mutants.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used were all derivatives of *E. coli* K-12. The genotypes of these strains are given in Table 1. Strain CY149 was derived from strain CY148 by treatment with phage λvir. A broth culture of strain CY148 at 10^9/ml was inoculated with 5×10^8 λvir phage/ml and grown overnight at 37 C. The resulting culture of lambda-resistant bacteria was streaked onto a maltose-supplemented eosin-methylene blue plate. *mal*$^-$ colonies were then tested for inability to grow on maltose as sole carbon source.

Those *mal*$^-$ colonies that were converted to *mal*$^+$ upon mating with strain F141/JC1553 were considered *malA* (rather than *malB*) mutants. Strain CY150 is a *recAl thy*$^+$ *str*$^-$ recombinant of a cross between Hfr KL16-99 and strain CY149. Inheritance of the *recAl* allele was scored by sensitivity to ultraviolet light (200 ergs/mm^2).

TABLE 1. *Bacterial strains*

Strain	Sex	Genotype[a]	Reference
8	HfrC	*glpD3, glpR2, phoA8, tonA22, rel-1* (λ)	2
BB13	HfrC	*plsB13*, other markers as in strain 8	2
BB26	HfrC	*plsB26*, other markers as in strain 8	2
BB26-36	HfrC	*plsB26*, mutation allowing supplementation with glycerol; other markers as in strain 8	2
KL185	F⁻	*pyrD34, thi-1, his-68, trp-45, galK35, xyl-7, mtl-2, str-118, malA1*, λ⁻	K. B. Low strain
GN1908	F⁻	*pyrD34, thi-1, his-68, thyA25, galK35, xyl-7, mtl-2, glpD3, glpR2, str-118, phoA8, glpk4*, λ⁻	G. N. Godson (10), formerly called 1908 (ref. 6)
CY148	F⁻	*plsB13, glpD3, glpR2, thyA25, thi-1, galK35, his-68, str-118, xyl⁺, mtl⁺*	Recombinant of cross of BB13 with GN1908
CY149	F⁻	*malA*⁻ derivative of CY148	See text
CY150	F⁻	*thy⁺, recA1* derivative of CY149	See text
KL16-99	Hfr	*recA1, rel-1, thi-1, drm-3*, λ⁻	K. B. Low strain
CY135	Hfr	*malA*⁻ derivative of BB13	See text
CY141	HfrC	*glpD11, glpR⁺, malA⁺* derivative of CY135	See text
CY142	HfrC	*malA⁺* derivative of CY135	See text
228	HfrC	*glpD11, glpR⁺, phoA8, tonA22, rel-1*, (λ)	E. C. C. Lin strain (4)
CBT312	F⁻	*thi-1, dctA2, sdh-2*	B. D. Sanwal (16)
KLF2/JC1553	F′	F102 *argG⁺/argG6, metB1, his-1, leu-6, recA1, mtl-2, xyl-7, malA1, gal-6, lacY1* or *Z4, str-104, supE44, tonA2, tsx-1* λ⁻	K. B. Low strain
KLF41/JC1553	F′	F141 *argG⁺*/(as above)	K. B. Low strain
MAF1/JC1533	F′	F140 *argG⁺*/(as above)	W. Maas strain
KLF11/JC1553	F′	F111 *metB⁺*/(as above)	K. B. Low strain
KL25, KL228	Hfrs	See Low (17, 18) for genotypes	K. B. Low strain
KL14, EC2, KL16		See Fig. 1 for origins of transfer	

[a] The allele numbers are those of the coli Genetic Stock Center, Yale University. The genetic symbols are those of Taylor and Trotter (26). All strain 8 derivatives are resistant to phage T_2. All the *malA*⁻ strains are resistant to phage λ.

Strains CY141 and CY142 were constructed starting with strain BB13. Strain CY135, a *malA* λR derivative of strain BB13, was transduced with a P_1 phage stock grown on strain 228. The phage-bacteria mixture was plated at 42 C on minimal maltose plates supplemented with G3P. Of the maltose-positive transductants, none were able to grow on G3P as carbon source at 42 C, but 22 grew on this medium at 30 C. All *mal⁺* transductants tested still required supplementation with G3P for growth on glucose minimal medium. Two transductants, strains CY141 and CY142, were selected for further study. Strain CY141 is able to use G3P as carbon source at 30 C, whereas strain CY142 is unable to grow on this medium at any temperature tested.

Media. The media used for genetic mapping (5–7) and for enzyme preparation (5) have been described previously. Sodium *rac*-G3P was the G3P source. The maltose-eosin-methylene blue medium is the EMBO recipe of Gottesman and Yarmolinsky (11) plus 0.5% maltose.

Enzyme assays. *sn*-G3P acyltransferase activity was assayed as previously described (2) with palmityl-coenzyme A as the acyl donor.

Genetic crosses. The procedures for conjugational and transductional crosses were described previously (5–8). Transductions in which *dctA*⁻ was an unselected marker were usually selected on lactate as carbon source and scored by comparison of their growth on plates containing either lactate or malate as carbon source.

RESULTS

The enzymatic defect is known for two classes of G3P auxotrophs (2). The class dealt with in this paper is called *plsB* (for phospholipid synthesis). The *plsB* mutants owe their G3P requirement to a defect in G3P acyltransferase, the first phospholipid biosynthetic enzyme. The K_m for G3P utilization by the mutant acyltransferases is >10-fold above the K_m of the wild-type enzyme (2). The other class of auxotroph (called *gpsA*) is due to a lesion in the structural gene for the biosynthetic G3P dehydrogenase and was previously mapped (5).

Identity of the plsB phenotype and the acyltransferase defect. Bell (2) had previously shown that the G3P acyltransferase activity of a class of G3P auxotrophs has a greatly increased K_m for G3P. It was shown that revertants to

G3P prototrophy regain acyltransferase activity with a normal K_m for G3P (2). We have now tested two *plsB*$^+$ transductants and a *plsB*$^-$ recombinant (strain CY148). As shown in Table 2, the *plsB*$^+$ transductants have a normal K_m for G3P, whereas the *plsB*$^-$ recombinant has a K_m about 10-fold higher than the parental strain. Therefore, the *plsB* locus and the acyltransferase defect act as a single genetic entity.

General mapping of plsB. The *plsB*$^-$ strains were isolated in a male strain. Hence, to obtain a *plsB*$^-$ female derivative, strain BB13 was mated with several multiply auxotrophic female strains, and *plsB*$^-$ was scored as an unselected marker. The most frequent inheritance of *plsB*$^-$ occurred when various amino acid and pyrimidine markers in the 60 to 80 minute region of the genetic map were selected. These results suggested that *plsB* was located in this region of the genetic map. This was confirmed by mating strain CY148, a *xyl*$^+$ *plsB*$^-$ recombinant of a mating between strains BB13 and GN1908, with various Hfr donor strains (Table 3). Large numbers of recombinants were formed only in matings with Hfr strains KL228, EC2, and KL14. Considering the origins of transfer of the various Hfr strains tested (Fig. 1), *plsB* must lie between the origins of Hfr strains KL228 and EC2. Similar results were obtained using "F$^-$ phenocopy" cultures of a *str*$^-$ derivative of strain BB13. Approximately equal numbers of *plsB*$^+$ recombinants were formed when the Hfr strain was counterselected by streptomycin or by omission of nutrients required by the Hfr strain. This finding suggested that *plsB* and *strA* are not closely linked. Furthermore, since the genes from *bioH* to *asd* can be deleted without engendering a G3P requirement (4, 24), *plsB* could not be in this region. Therefore, *plsB* was thought to be close to the *mtl* region of the genetic map. It should be noted that inheritance of *plsB*$^-$ as an unselected marker was either very poor or nonexistent when *xyl*$^+$, or *mtl*$^+$, or *glpD*$^+$ recombinants were selected (even though *plsB* is located within a few minutes of these markers). However, in experiments in which any of several amino acid or nucleic acid base markers was the selected marker, *plsB*$^+$ was found as the unselected marker at a normal frequency. Such effects are not limited to *plsB*$^+$, since selection of *mtl*$^+$ (but not *xyl*$^+$) recombinants in crosses of *mtl*$^-$ *gpsA*$^+$ strains yield no *mtl*$^+$ *gpsA*$^-$ strains. We have no explanation for this behavior.

TABLE 2. *Properties of G3P acyltransferase preparations from various strains*

Strain	*plsB* phenotype	K_m for G3P (μM)[a]
Strain 8	+	88
BB26-36	−	1,000
BB26-36 T1[b]	+	90
BB26-36 T2[c]	+	85
BB13	−	1,250
CY148[d]	−	950

[a] The K_m values were derived from Lineweaver-Burk plots as previously described (2).

[b] *plsB*$^+$ *dctA*$^-$ transductant from cross 5 in Table 5.

[c] *plsB*$^+$ transductant from cross 6 in Table 5.

[d] Strain CY148 was prepared by mating strain GN1908 with strain BB13. Strain GN1908 was prepared by mating strain KL190 with strain 8 (10). Therefore, strain 8 seems an appropriate wild-type strain for comparison with strain CY148.

TABLE 3. *Localization of the plsB locus by conjugation*

Recipient	Hfr donor (*plsB*$^+$ *str*$^-$ recombinants/ml)				
	KL16	KL228	KL14	EC2	KL25
CY148	<10	5×10^6	5×10^5	2.3×10^5	<10
BB13 *str*$^-$[a]	<10	2×10^5	1.6×10^5		<10[3]

[a] An F$^-$ phenocopy culture was the recipient. The phenocopy culture was obtained by starvation of long-phase strain BB13 *str*$^-$ cells in medium E without carbon source for 5 h at 37 C (21).

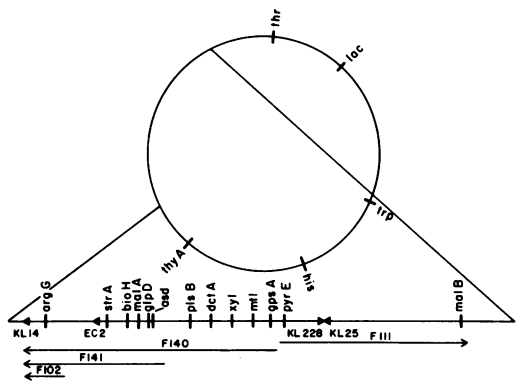

FIG. 1. *Genetic map of E. coli K-12. The distances are drawn to approximate scale. Most of the data is from Taylor and Trotter (26). The Hfr and F' data are from Low (17). The data for the gpsA locus were reported previously (5).*

Mapping with F' donor strains. Finer mapping of the *plsB* locus was performed using several F' donor strains (Fig. 1). Four derivatives of strain JC1553, each carrying a different F' factor, were mated with strains CY149 and CY150. The donor strain was *recA*$^-$, thus preventing chromosome mobilization. Only transfer of the F140 episome gave rise to *plsB*$^+$ recombinants in matings with either strain

CY149 or with strain CY150, a *recA*$^-$ derivative of strain CY149 (Table 4). Strains carrying F141, F111, and F102 did not donate *plsB*$^+$ to either recipient strain, although the donor cultures were fully fertile. Therefore, the *plsB*$^+$ locus lies between the termini of the chromosomal portions carried by F141 and F140 and thus is located between minute 66 and 71 of the genetic map (Fig. 1).

Similar numbers of *plsB*$^+$ recombinants were formed when F140/JC1553 was mated with either a *recA*$^+$ or a *recA*$^-$ recipient strain (Table 4). This result indicates that *plsB*$^-$ is recessive to *plsB*$^+$, a result consistent with the in vitro data (2). It should be noted that crosses of F140 with *malA pls B* recipients resulted in formation of significantly more *malA*$^+$ recombinants than *plsB*$^+$ recombinants. We attribute this finding to the known frequent occurrence of variants of F140 from which parts of the F′ have been deleted (13, 17; B. Low, personal communication). This behavior precludes complementation analysis of *plsB* using this F′.

Transductional mapping. In order to localize the *plsB* locus further, we tested for cotransduction by phage P_1 of *plsB* with other markers in this chromosomal segment. We transduced strains BB26-36 and BB13 to *plsB*$^+$ with phage stocks grown on strains mutant in the appropriate genes and tested for the unselected marker. *plsB*$^-$ was not used as an unselected marker owing to its anomalous behavior as an unselected marker in conjugational crosses. As shown in Table 5, <0.25% cotransduction was observed with the *xyl*, *mtl*, *glpD*, *malA*, or *asd* loci. However, cotransduction was observed with the *dctA* (formerly called *dct*) locus (16). Mutants in the *dctA* locus are isolated as being resistant to 3-fluoromalate and hence defective in transport of C_4 dicarboxylic acids (14, 16). Kay and Kornberg (14) have mapped this locus just counterclockwise of the *xyl* locus. Unfortunately, *dctA*$^-$ strains are extremely unstable (H. Kornberg and B. D. Sanwal, personal communications) and thus cannot be used as a selected marker in transductional crosses. In fact, *dctA* is a poor nonselected marker, since *plsB*$^+$ *dctA*$^-$ recombinants were often observed to revert to *dctA*$^+$ upon prolonged incubation. Three *dctA* strains were used in this work. P_1 phage stocks propagated on strain CBT12 gave about 14% cotransduction when used to form *plsB*$^+$ recombinants. Phage stocks from the two other strains gave lower cotransduction frequencies (1 to 3%), but these strains reverted to *dctA*$^+$ even more often than strain CBT12. The instability of the *dctA* strains probably means that the cotransduction frequencies observed are an underestimation of the true frequency.

The finding that *plsB* is cotransduced with *dctA* but not with *xyl* or *mtl* indicates that *plsB* is located on the side of *dctA* distal to *xyl*. However, *plsB* is not cotransduced with the *asd* locus, thus indicating that the distance between *asd* and *dctA* is not less than that given by Taylor and Trotter (26). Therefore, the clockwise map order of *asd*, *plsB*, *dctA*, *xyl*, *mtl* is indicated for this region of the linkage map. The final results of the mapping experiments are given in Fig. 1.

Recombination between plsB mutants. Bell (2) isolated a number of independently derived mutants with K_m defects in the acyltransferase. We have asked whether all these mutants are located in the immediate vicinity of the *plsB13* allele. No *plsB*$^+$ *str*$^-$ recombinants are found in crosses between various *plsB* strains and strain

TABLE 4. *Mapping of plsB by F′ transfer*

Episome[a]	Recipient	*recA*	Recombinants/ml	
			plsB$^+$	*malA*$^+$
F140	CY149	+	4.8×10^4	6.4×10^6
F140	CY150	−	6.0×10^4	5.2×10^6
F141	CY149	+	<100	4.0×10^6
F141	CY150	−	<100	4.4×10^6
F102[b]	CY150	−	<100	
F111[c]	CY150	−	<100	

[a] The episomes were all transferred from male JC1553 derivatives. The donor strains were counterselected by omission of leucine, arginine, and methionine from the selection medium.

[b] This culture transferred *argG*$^+$ to strain KL141 (5) (efficiency of about 0.1 per male cell).

[c] This culture transferred *metE*$^+$ to strain CY123 (5) at an efficiency of about 0.001 per male cell.

TABLE 5. *Transductional mapping of the plsB locus*[a]

Cross no.	Donor	*plsB*$^+$ colonies with donor marker/total *plsB*$^+$ colonies scored	Cotransduction frequency %
1	KL185 *xyl*$^-$	0/405	<0.25
2	KL185 *mtl*$^-$	0/405	<0.25
3	KL185 *malA*$^-$	0/399	<0.25
4	KL185 *glpD*$^+$	0/405	<0.25
5	U482 *asd*$^-$	0/780	<0.13
6	CBT312 *dctA*$^-$	44/312	14.1

[a] Strain BB26-36 was the recipient used in these crosses. Crosses identical to 1-4 were also performed with strain BB13. These crosses gave <0.39% cotransduction for these markers with *plsB*.

CY149 (Table 6). However, *malA*$^+$ recombinants are formed at a normal frequency. This result indicates that the *plsB* alleles present in these strains are located very close to the *plsB13* allele and thus are probably mutations within the same genetic locus defined by *plsB13*.

Strain K956 in Table 6 is the mutant isolated by Kito et al. (15). This strain grows slowly without G3P and normally in the presence of G3P. This phenotype is believed to be due to a K_m defect in the acyltransferase (15, 22). Is this strain a *plsB* mutant? The experiment in Table 6 gives equivocal results to this question. Strain K956 gives no *plsB*$^+$ recombinants when crossed with strain CY149. This result was unexpected. If strain K956 was defective only in the *plsB* gene, then a number of small colonies (comparable to the number of *malA*$^+$ recombinants) should have been formed. These colonies should have the "leaky" G3P requirement characteristic of strain K956. No such colonies were found. This finding suggests that strain K956 owes its phenotype to at least two mutations. This suggestion seems likely since Pizer and co-workers (22) have already found that strain K956 differs from its parent in its *rel* gene phenotype as well as in its requirement for G3P.

Functional independence of plsB$^-$ and glpD$^-$. The *plsB*$^-$ mutants studied were isolated in a *glpD*$^-$ strain. This background was chosen since *glpD*$^-$ strains accumulate abnormally high intracellular pools of G3P (12). Since the *plsB* mutants owe their requirement for G3P to a K_m defect in the acyltransferase, it was supposed that a *glpD*$^-$ lesion might be necessary to allow effective supplementation of *plsB* mutants with G3P by elevation of the intracellular pool. Perhaps in a normal *glpD*$^+$ strain exogenous supplementation would not result in a G3P pool sufficient to overcome the acyltransferase K_m defect. This could explain the genetic anomaly mentioned above. That is, when a *plsB*$^+$ *glpD*$^+$ Hfr strain is crossed with a *glpD*$^-$ *plsB*$^-$ female strain, no *glpD*$^+$ *plsB*$^-$ recombinants are formed, although *glpD*$^+$ *plsB*$^+$, *plsB*$^+$ *glpD*$^-$, and *glpD*$^-$ *plsB*$^+$ recombinants occur frequently.

In order to test this hypothesis, we prepared strains CY141 and CY142. Strain CY141 is *glpD*$^-$ only at 42 C; at lower temperatures the strain is *glpD*$^+$. Strain CY142 is nearly isogenic to strain CY141 but has a *glpD* lesion which is unaffected by temperature. Both strains also carry a lesion in the *plsB* gene. Therefore, if the *plsB*$^-$ phenotype is viable only in a *glpD*$^-$ background, strain CY141 should have a cold-sensitive phenotype, compared to strain CY142. However, strain CY141 is not cold sensitive. This strain grows normally when it is *glpD*$^+$ either in short term (Fig. 2) or prolonged (Table 7) experiments. Therefore, a lesion in the *glpD* gene is not required for effective supplementation of *plsB*$^-$ strains with G3P.

TABLE 6. *Recombination between plsB mutants*

Strain[b]	Apparent K_m for G3P (μM)[c]	Recombinants with CY149[a]	
		plsB$^+$ *str*$^-$	*malA*$^+$ *str*$^-$
BB13	1,250	<0.002	1.7
1A4	1,050	<0.002	1.1
BB26-36	1,000	<0.002	1.6
BB29	850	<0.002	2.1
BB30	900	<0.002	1.7
K956[d]	374[e]	<0.002	1.3

[a] To be multiplied by 10^6.

[b] Those strains not listed in Table 1 have genotypes identical to that of strain BB13 except for the *plsB* allele. Strain 8, the wild-type *plsB*$^+$ parent, has a G3P K_m of 90 μm.

[c] The K_m values were determined from Lineweaver-Burk plots as previously described (2).

[d] Since strain K956 has only a "leaky" requirement for G3P (15, 22), about 10^6 small *plsB*$^+$ colonies were expected; however, no such colonies were observed.

DISCUSSION

G3P acyltransferase catalyzes the first step of phospholipid biosynthesis, the formation of lysophosphatidic acid (19, 20, 22, 23, 25, 27). This enzyme activity is located on the cytoplasmic (inner) membrane of the *E. coli* cell envelope (3, 29). Two lines of indirect evidence indicate that phospholipid is required for G3P acyltransferase activity. Treatment of cell en-

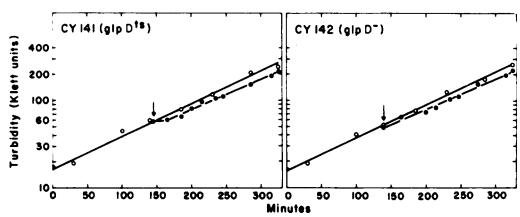

FIG. 2. *Growth of strains CY141 (left panel) and CY142 (right panel) at various temperatures. The strains were grown in the low phosphate (1 mM) medium previously described (2) supplemented with glucose (0.4%), rac-G3P (0.1%), and casein hydrolysate (1.0%) at 42 C. At the time indicated by the arrow, a portion of each culture was shifted to a 35 C water bath. The growth was followed with a Klett colorimeter with a green filter. A reading of 20 is equal to about 10^8 cells/ml.*

TABLE 7. *Growth[a] of strains CY141 and CY142*

Carbon source		G3P supplement	Incubation temp (C)	Strain	
Glucose	G3P			CY141	CY142
+		+	42	4+	4+
+		0	42	0	0
	+		42	0	0
+		+	37	4+	4+
+		0	37	0	0
	+		37	3+	0
+		+	30	3+	3+
+		0	30	0	0
	+		30	2+	0
+		+	25	2+	2+
	+		25	+	0

[a] These tests were performed on solid medium E (28) plates supplemented with 0.4% glucose or 0.4% G3P as carbon source plus or minus 0.05% G3P as supplement. Strains CY141 and CY142 were inoculated onto the same plate for each test. The number of plus signs denotes colony size (4+ = ca. 4 mm in diameter). The plates were incubated for 48 to 72 h (depending on the culture condition) before scoring.

velopes with pure phospholipase C abolishes G3P acyltransferase activity (19). Also, the activity of the enzyme at various temperatures can be altered by manipulation of the fatty acid composition of the envelope phospholipids (20). The in vitro specificity of the G3P acyltransferase is marked (23, 25, 27). Unsaturated fatty acids are transferred to position 2 of the G3P molecule, whereas palmitate is acylated to position 1 (23, 25, 27). In accord with the in vivo situation, this specificity is altered by a change in incubation temperature (25). Therefore, G3P acyltransferase is a very complex enzyme activity, and the genetic analysis of mutation affecting this activity could also be complex.

Two classes of mutants defective in G3P acyltransferase have been isolated. The *plsB* mutants mapped in this paper have a 10-fold elevated K_m for G3P. The *plsA* mutants have a ts defect in the acyltransferase activity (6, 23). We have now mapped the *plsB* locus at minute 69 of the *E. coli* genetic map, whereas the *plsA* mutants (6) were previously located at minute 13, one-third of the map distance removed from *plsB*. Both types of mutants appear to be single mutational lesions and are recessive to their wild-type alleles. The demonstration that *plsA* and *plsB* are separate genetic entities allows several explanations for the different effects on growth and macromolecule synthesis seen with these two mutant types.

It is possible that the G3P acyltransferase is a multienzyme complex or an enzyme composed of nonidentical subunits. Therefore, the *plsA* and *plsB* genes could code for separate parts of the active enzyme. In such cases, the genes coding the components are usually closely linked, which *plsA* and *plsB* are not. However, exceptions in which genes coding components of a complex are not linked, such as in the α-ketoglutaric dehydrogenase complex (1) of *E. coli*, are known. If one of the gene products is also involved in other metabolic processes this could explain the differing phenotypes observed under nonpermissive conditions.

Another possibility is that neither *plsA* or *plsB* is a structural gene for an acyltransferase component, but codes for another membrane component which interacts with the acyltransferase. A separate membrane protein juxtaposed to the acyltransferase could produce either the *plsA* or the *plsB* phenotype or both. Resolution of these hypotheses must await solubilization and purification of the G3P acyltransferase from the cytoplasmic membrane. Unfortunately, numerous attempts in several laboratories have not succeeded in this aim. We are currently preparing *plsA plsB* double mutants in order to study the interaction of these two phenotypes in vivo and in vitro.

ACKNOWLEDGMENTS

We thank B. Bachmann, L. Pizer, and B. Low for numerous gifts of strains and L. Pizer for unpublished information on his strains.

This work was supported by National Science Foundation grants GB-32063 to J. Cronan and GB-38382 to R. Bell and Public Health Service grant AI-10186 from the National Institute of Allergy and Infectious Disease to J. Cronan. J. Cronan is supported by Public Health Service Research Career Development Award GM-70411 from the National Institute of General Medical Sciences.

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