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Mutants of *Escherichia coli* Defective in Membrane Phospholipid Synthesis: Mapping of the Structural Gene for L-Glycerol 3-Phosphate Dehydrogenase

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The structural gene for the biosynthetic L-glycerol 3-phosphate dehydrogenase has been mapped at min 71.5 on the *Escherichia coli* chromosome. This gene (*gpsA*) is co-transduced with the *xyl*, *mtl*, and *pyrE* loci. Three-factor conjugational crosses and the transduction data indicate that the order of loci in this region of the chromosone is *mtl*, *gltE*, *gpsA*, *gadR*, *gadS*, *pyrE*. Study of a temperature-sensitive *gpsA* mutant possessing a dehydrogenase of increased thermolability indicated that *gpsA* is the structural gene for the dehydrogenase. All dehydrogenase-deficient strains tested were mapped very close to the *gpsA* locus. Attempts at genetic complementation analysis were unsuccessful.

The availability of *Escherichia coli* mutants deficient in membrane phospholipid synthesis has facilitated investigations on the structural and functional importance of phospholipids in biological membranes (5). L-Glycerol 3-phosphate (identical to *sn*-glycero-3-phosphate) is a precursor required for the synthesis of all the phospholipid species found in *E. coli* (5). The synthesis of this phospholipid precursor was first studied by Kito and Pizer (10). These workers described an enzyme from *E. coli* that reduced dihydroxyacetone phosphate to L-glycerol 3-phosphate. This enzyme was shown to be distinct from the L-glycerol 3-phosphate dehydrogenase involved in the aerobic catabolism of glycerol (6) and was strongly inhibited by L-glycerol 3-phosphate (10). These data led Kito and Pizer (10) to propose that this enzyme is responsible for the synthesis of the L-glycerol 3-phosphate needed as a phospholipid precursor.

This proposal was confirmed by the isolation of mutants deficient in this enzyme activity first by Hsu and Fox (8) and later by Bell (1). These mutants were selected as either glycerol (8) or L-glycerol 3-phosphate (1) auxotrophs of *E. coli* K-12 and were found to be defective in phospholipid synthesis when deprived of the required supplement. In this paper, we report the genetic mapping of the class of L-glycerol 3-phosphate auxotrophs deficient in the biosynthetic L-glycerol 3-phosphate dehydrogenase and demonstrate that the genetic locus (*gpsA*) defined by these mutants is the structural gene for the enzyme.

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 CY123 was constructed by mating strain KL163 with strain CY115. After mating for 30 min at 37 C, the mating mixture was diluted 100-fold into minimal medium supplemented with glucose, streptomycin, methionine, and tryptophan. This culture was grown overnight and then plated on broth plates supplemented with 10 μg of naladixic acid per ml of medium. Small colonies were picked and checked for sensitivity to ultraviolet (UV) light (200 ergs/mm^2). Strain CY123 is one such UV^S Nal^R recombinant.

Temperature-sensitive mutants which contained a thermolabile L-glycerol 3-phosphate dehydrogenase were selected by screening mutagen-induced (1) revertants of strain BB20-14 for those capable of prototrophic growth at 25 C but unable to grow at 42 C unless the medium was supplemented with L-glycerol 3-phosphate. Twelve of the 563 revertants tested showed this property. One of these strains, BB20-14-10d8, was selected for further investigation. This strain, like its parent (1), had no nutritional requirements other than L-glycerol 3-phosphate or glycerol, since it grew well on minimal glucose plates at 25 C and on minimal glucose plates supplemented with either glycerol or DL-glycerol-3-phosphate at 42 C. The reversion rate to temperature resistance was about 10^{-8} reversions per generation per bacterium.

Media and growth of cells. The media used for genetic studies have been described previously (4). Carbon sources were added to 0.2%. Sodium DL-glycerol 3-phosphate was added to 0.1%. The growth temperature was 37 C unless indicated otherwise.

Cultures for preparation of enzyme extracts were grown in medium E (20) supplemented with 0.3%

each of Casamino Acids (vitamin free), yeast extract, and nutrient broth. Glucose (0.2%), DL-glycerol 3-phosphate (0.1%), and tryptophan uracil, thymine, and guanosine (each at 20 mg/liter of medium) were also included in this medium. Overnight cultures were harvested by centrifugation, washed with distilled water, and frozen.

L-Glycerol 3-phosphate dehydrogenase assay. L-Glycerol 3-phosphate dehydrogenase activity was assayed at 25 C spectrophotometrically in crude extracts as described previously (1, 10). One unit of activity corresponds to the dihydroxyacetone phosphate-dependent oxidation of 1 nmol of reduced nicotinamide adenine dinucleotide phosphate per min at 25 C.

Genetic crosses. The procedures for conjugational

TABLE 1. *Bacterial strains*

Strain	Sex	Genotype[a]	Source or reference
Strain 8	HfrC	*glpD3, glpR^{c}2, phoA8, tonA22, T2^R, rel-1,* (λ)	E. C. C. Lin (6)
BB20	HfrC	*gpsA20, glpD3, glpR^{c}2, phoA8, tonA22, T2^R, rel-1* (λ)	1
BB20-14	HfrC	Same as BB20 except able to utilize glycerol as supplement presumably due to a glycerol kinase resistant to fructose 1,6-diphosphate	1
BB20-14-10d8	HfrC	Same as BB20-14 except *gpsA1* rather than *gpsA20*	See text
X478	F$^-$	*proC32, purE42, metE70, lysA23, thi-1, leu-6, trpE38, lacZ36, mtl-1, xyl-5, ara-14, azi-6, tonA23, tsx-67, str-109*	R. Curtiss strain
KL185	F$^-$	*pyrD34, thi-1, his-68, trp-45, gal-35, xyl-7, mtl-2, str-118*	K. B. Low strain
UC1	F$^+$	*str-146,* λ$^-$	4
CY115	F$^-$	*gpsA20, metE70, trpE-8, xyl-5, tsx-67, str-109, mtl$^+$, proC$^+$, purE$^+$, leu$^+$, lys$^+$* (other markers unchecked)	See text
CY119	HfrC	Same as BB20 except *gpsA$^+$, mtl-2*	*gpsA$^+$, mtl$^-$* transductant of BB20 by KL185
CY123	F$^-$	Same as CY115 except *nalA12, recA1*	See text
KL163	Hfr	*recA1, nalA12, thyA25, rel-1*	K. B. Low strain
KL141	F$^-$	*pyrE41, argG6, thyA25, malA1, rbs-1, strA8 or 17 or 104*	K. B. Low strain
CS101-4U	HfrC	*pyrE41, metB1, tonA22, rel-1, T2^R*	AT2243, A. L. Taylor strain (18)
EM111	F$^-$	*gltE2, thi-1, ilv-275, mtl-1, xyl-5, ara-14, galK2, lacY1, tfr-5, tsx-57, str-20, supE44*	E. Murgola (14, 15)
MAF1/JC1553	F′	F140 *argG$^+$/argG6, metB1, his-1, leu-6, recA1, mtl-2, xyl-7, malA1, gal-6, lacY1,* or *Z4, str-104, sup-59, tonA2,* λ^R, λ$^-$	K. B. Low strain
KLF11/JC1553	F′	F111 *metB$^+$*/as above	K. B. Low strain
KL25, KL228, KL209, KL14, P4X (BW113), KL16	Hfr	See Low (12) for genotypes, see Fig. 2 for origins of transfer	K. B. Low strains

[a] The genetic nomeclature used is that of Taylor and Trotter (19). The allele numbers are those of the Coli Genetic Stock Center, Yale University.

and transductional crosses were described previously (3, 4). In crosses in which *gpsA*$^-$ was to be an unselected marker, the recipient was grown in broth plus 1% DL-glycerol 3-phosphate. This procedure greatly increased the inheritance of *gpsA*$^-$, presumably by inducing the L-glycerol 3-phosphate transport system (6) of the recipient.

RESULTS

Two different classes of L-glycerol 3-phosphate auxotrophs are known. One class, which we will call *gpsA* (for glycerol phosphate synthesis), is deficient in the biosynthetic L-glycerol 3-phosphate dehydrogenase (1, 8). The other class requires L-glycerol 3-phosphate due to a defect in the L-glycerol 3-phosphate acyltransferase (1, 9). This paper deals with only the former class of mutants.

Identity of gpsA phenotype and dehydrogenase deficiency. The biosynthetic L-glycerol 3-phosphate dehydrogenase activity and the *gpsA* locus act as a single genetic entity. Revertants to L-glycerol 3-phosphate prototrophy regain dehydrogenase activity (1). We tested both *gpsA*$^+$ and *gpsA*$^-$ transductants for dehydrogenase activity and found that four *gpsA*$^+$ transductants (two each from crosses 3 and 5 in Table 5) possessed the same level of activity as their *gpsA*$^+$ parents (which varied between 11 and 21 U/mg of protein), whereas four *gpsA*$^-$ transductants (from cross 6 in Table 5) had no detectable activity (<0.3 U/mg of protein).

Isolation and biochemical characterization of temperature-sensitive L-glycerol 3-phosphate dehydrogenase mutants. The genetic data presented above suggested that *gpsA* may be the structural gene for the biosynthetic L-glycerol 3-phosphate dehydrogenase. We confirmed this suggestion by the isolation of a temperature-sensitive L-glycerol 3-phosphate auxotroph that contains an L-glycerol 3-phosphate dehydrogenase activity of increased thermolability. This mutant, BB20-14-10d8, was isolated as a revertant of strain BB20-14 able to grow at 25 C without supplement but requiring DL-glycerol 3-phosphate or glycerol for growth at temperatures above 37 C. Extracts prepared from strain BB20-14-10d8 grown at 25 C contained L-glycerol 3-phosphate dehydrogenase activity comparable to that of strain 8 (Table 2), but extracts prepared from strain BB20-14-10d8 incubated at 42 C contained no detectable dehydrogenase activity. Strain BB20-14, the immediate parent of strain BB20-14-10d8, had no detectable enzyme activity in extracts from cells grown at either temperature (Table 2). Therefore, it seemed apparent that strain BB20-14-10d8 phenotype was caused by a missense mutation which resulted in the production of a thermolabile L-glycerol 3-phosphate dehydrogenase. Indeed, the L-glycerol 3-phosphate dehydrogenase activity of strain BB20-14-10d8 is much more thermolabile than that of strain 8. Extracts of the mutant culture incubated at elevated temperatures for various periods of time prior to assay at 25 C lost activity more rapidly than the wild-type extracts at all temperatures tested (Fig. 1). When a strain BB20-14-10d8 extract that had been heated (to inactivate L-glycerol 3-phosphate dehydrogenase activity) was mixed with a wild-type extract, no inhibition of the wild-type activity occurred. Thus, the increased thermolability is not caused by a temperature-induced inhibitor.

TABLE 2. *L-Glycerol 3-phosphate dehydrogenase activity in parental, mutant, and revertant extracts*

Strain	Temp of growth (C)	L-Glycerol 3-phosphate dehydrogenase sp act[a]
8	25	26.1
8	42	18.7
BB20-14	25	<0.3
BB20-14	42	<0.3
BB20-14-10d8	25	16.0
BB20-14-10d8	42[b]	<0.3
BB20-14-10d8 R1[c]	42	17.2
BB20-14-10d8 R3[c]	42	16.6
BB20-14-10d8 R5[c]	42	9.8

[a] Activity is expressed in units per milligram of protein.

[b] Cultures growing exponentially at 25 C in a glycerol-supplemented medium were shifted to 42 C for 16 h prior to harvest.

[c] R signifies revertant to temperature resistance.

The temperature-sensitive lesion in strain BB20-14-10d8 has a reversion rate of about 10^{-8} reversion to temperature resistance per generation per bacterium and, thus, appears due to a single mutational event. Extracts prepared from three spontaneous *gpsA*$^+$ revertants of strain BB20-14-10d8 contained nearly wild-type levels of L-glycerol 3-phosphate dehydrogenase activity (Table 2). All of the revertants examined contained an L-glycerol 3-phosphate dehydrogenase activity much less thermolabile than strain BB20-14-10d8 (Table 3). The temperature-sensitive mutation in strain BB20-14-10d8 recombined at an extremely low rate with the *gpsA20* allele and, thus, is almost certainly a lesion in the *gpsA* locus (see Table 8).

Conjugational mapping of gpsA. Since the *gpsA* mutants were isolated in a male strain, we

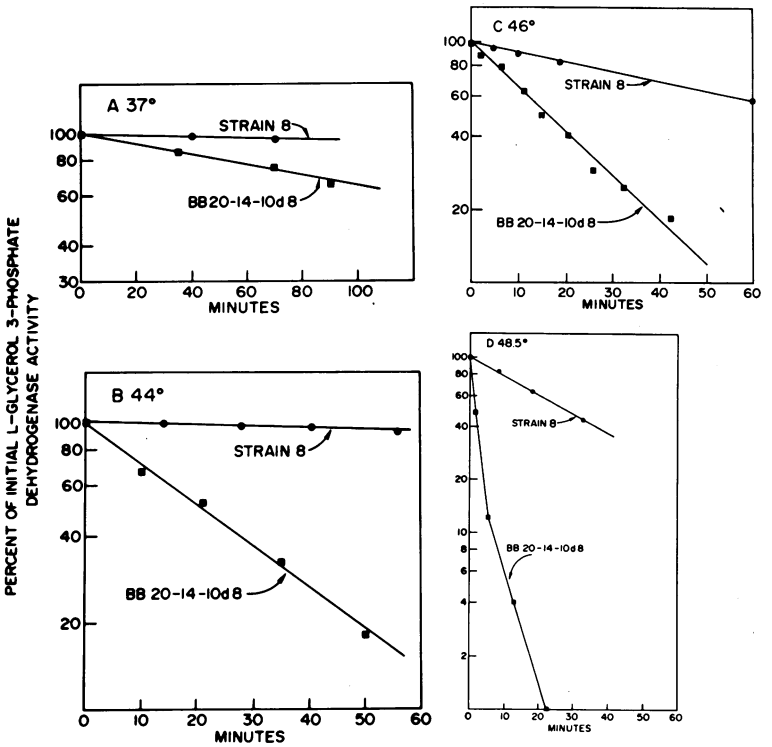

FIG. 1. *Thermolability of the L-glycerol 3-phosphate dehydrogenase activities of strain 8 and the temperature-sensitive L-glycerol 3-phosphate auxotroph BB20-14-10d8. Crude extracts of strain 8 and BB20-14-10d8 were prepared from cells grown at 25 C as described previously (1). A portion of each extract was incubated at the temperature indicated. Samples were removed at various times and assayed for L-glycerol 3-phosphate dehydrogenase activity at 25 C. The data are expressed relative to the unheated sample of the same extract. The specific activities of these extracts are given in Table 3.*

TABLE 3. *Thermolability of L-glycerol 3-phosphate dehydrogenase in parental, mutant, and revertant extracts*

Strain	Half-life of inactivation at 44 C (min)
8[a]	>300
BB20-14-10d8[a]	22
BB20-14-10d8 R1[b]	170
BB20-14-10d8 R3[b]	150
BB20-14-10d8 R5[b]	100

[a] Derived from data shown in Fig. 1.

[b] Extracts prepared from cells grown at 42 C. R signifies revertant to temperature resistance.

TABLE 4. *General mapping of gpsA*

Selected[a] recombinants	No. scored	*gpsA*⁻ recombinants (%)
proC⁺, *purE*⁺	65	0
leu⁺	61	0
metE⁺	55	5.5
lysA⁺	70	15.7
trpE⁺	81	1.2

[a] The donor was counterselected by streptomycin. The donor was strain BB20 (*str*^{S}, *gpsA*⁻). The recipient was strain X478 (*proC*⁻, *purE*⁻, *leu*⁻, *trpE*⁻, *str*^{R}).

first transferred the *gpsA20* marker to a female strain, X478. Various recombinants of the cross between strains BB20 and X478 were scored for inheritance of the *gpsA20* marker. *metE*⁺ and *lysA*⁺ recombinants received the *gpsA* marker of strain BB20 most frequently, thus positioning the *gpsA* locus in the vicinity of these genes (Table 4). One of the *lys*⁺ *gpsA*⁻ recombinants of this cross (CY115) was then mated with various Hfr strains with origins of transfer beginning in this region. These experiments showed that *gpsA*⁺ *str*^{R} recombinants were formed at high frequency (1 to 10% of donor input) in crosses with Hfr strains KL209, KL228, and KL14. Crosses with Hfr strains C and P4X gave lower numbers of recombinants, whereas crosses with strains KL25 and KL16 gave extremely few recombinants. Considering the origins of transfer of the Hfr strains (Fig. 2),

the conjugation data indicated that the *gpsA* locus lay between the origins of strains KL228 and KL14. Comparison of the frequency of linkage between *gpsA* and *metE* (32% linkage) and between *xyl* and *metE* (29% linkage) suggested that *gpsA* lay in the vicinity of the *xyl* and *mtl* loci.

Transductional mapping of gpsA. The accumulated data suggested that *gpsA* may be co-transduced with the *xyl* or *mtl* loci, or both. As shown in Table 5, *xyl* and *gpsA* were about 8% co-transducible (crosses 1 to 4) and *gpsA* and *mtl* were about 54% co-transducible (cross 5). Since Solomon and Lin (17) have shown that *mtl* and *xyl* are 15% co-transducible, this suggested the order of these genes was *xyl mtl gpsA*. Assuming this order, we tested for co-transduction of *gpsA* and *pyrE*, a marker on the side of *mtl* distal to *xyl*. We found that about 57% of *gpsA*$^+$ transductants also inherited *pyrE* (cross 6). The reciprocal cross gave a somewhat lower figure (cross 7). Schaefler and Maas (16) have found *pyrE* and *mtl* to be 13% co-transduced, somewhat less than the *pyrE-gpsA* frequency, again suggesting the order *xyl mtl gpsA*.

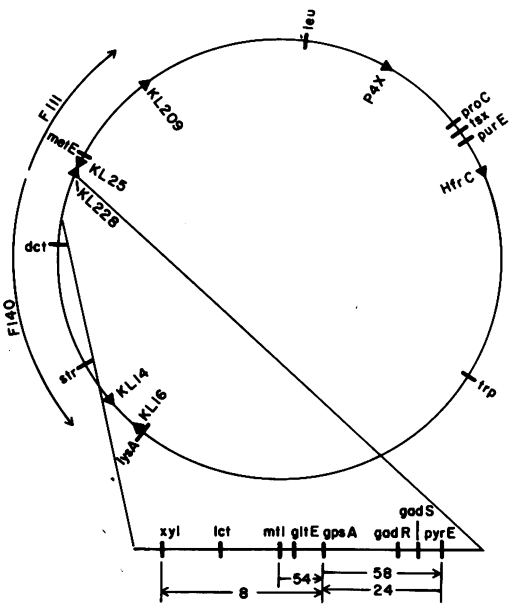

FIG. 2. *Location of gpsA on the genetic map of E. coli. The circular map at the top is adapted from that of Taylor and Trotter (19). The origins and directions of transfer of the Hfr strains and the regions carried by the F' strains are taken from Low (11, 12). The figures at the bottom are phage P_1 co-transduction frequencies from the data of Table 5. No attempt has been made to draw the enlarged portion of the figure to scale.*

Orientation of the mtl and gpsA loci. The order of the genes in this region could be either *xyl mtl gpsA* or *xyl gpsA mtl*. The genes were found to have the former order (that suggested by the transduction data) by linkage analysis of the pair of reciprocal three-factor conjugational crosses illustrated in Fig. 3.

If the order is *xyl, mtl, gpsA*, then *xyl*$^+$, *mtl*$^+$, *gpsA*$^+$ recombinants would be the result of double crossing-over when *gpsA*$^-$ is a donor marker (Fig. 3A) and of quadruple crossing-over when *gpsA*$^-$ is a recipient marker (Fig. 3B). The opposite result (Fig. 3C and D) is expected if the order is *xyl, gpsA, mtl*. Thus, by comparing the ratio of *xyl*$^+$, *mtl*$^+$, *gpsA*$^+$ recombinants to *xyl*$^+$ recombinants, the higher ratio should be pro-

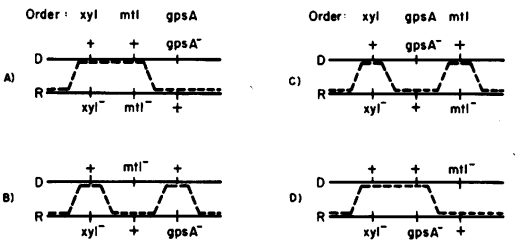

FIG. 3. *Possible arrangements of gpsA with respect to xyl and mtl in reciprocal crosses. The broken lines represent the crossing over necessary for the formation of gpsA*$^+$, *xyl*$^+$, *mtl*$^+$ *recombinants. Symbols: D, donor; and R, recipient.*

TABLE 5. *Transductional mapping of the gpsA locus*

Cross no.	Bacterial strains and relevant markers		Marker selected	Colonies with donor marker/ total colonies selected	Co-transduction frequency (%)
	Donor	Recipient			
1	KL185 (*xyl*$^-$)	BB20 (*gpsA*$^-$)	*gpsA*$^+$	8/154	5.2
2	X478 (*xyl*$^-$)	BB20 (*gpsA*$^-$)	*gpsA*$^+$	13/169	7.7
3	UC1 (*xyl*$^+$ *gpsA*$^+$)	CY115 (*xyl*$^-$ *gpsA*$^-$)	*gpsA*$^+$	31/354	8.8
4	UC1 (*xyl*$^+$ *gpsA*$^+$)	CY115 (*xyl*$^-$ *gpsA*$^-$)	*xyl*$^+$	6/129	4.6
5	KL185 (*mtl*$^-$)	BB20 (*gpsA*$^-$)	*gpsA*$^+$	83/154	53.9
6	BB20 (*gpsA*$^-$)	KL141 (*pyrE*$^-$)	*pyrE*$^+$	53/92	57.5
7	CS101-4U1 (*pyrE*$^-$)	BB20 (*gpsA*$^-$)	*gpsA*$^+$	31/132	23.5

duced by the order which depends on double crossing-over (Table 6). The proportion of *xyl*$^+$, *mtl*$^+$, *gpsA*$^+$ is clearly highest when *gpsA*$^-$ is the donor allele. This result is only consistent with the order *xyl*, *mtl*, *gpsA*. It should be noted that excessive recombination appears to occur in crosses in which *gpsA*$^-$ is a donor marker (see Table 7). This finding is probably due to a counter-selection against *gpsA*$^-$ in the recipient. However, this complication does not affect the conclusion that the map order is *xyl*, *mtl*, *gpsA*. The transduction data given above indicated that *gpsA* is between *mtl* and *pyrE*, which was confirmed by a three-factor conjugation cross between strains CS101 4U and CY115 (data not shown).

Other markers in the map region. Three additional markers are known to be located between the *mtl* and *pyrE* loci. Two of these, *gadS* and *gadR*, lie very close to *pyrE* (13). We did not examine the linkage of *gpsA* with these markers since these *gad* mutants can be detected only by enzyme assay (13). However, since the *gad* loci and the *pyrE* locus are about 80% co-transducible (13), it seems likely the *gad* loci are between *gpsA* and *pyrE*.

The third gene mapped in this region is the *gltE* gene. The *gltE* strains are streptomycin-suppressible mutants that have lesions between *mlt* and *pyrE* and are co-transduced with *xyl* with a frequency of 5% (14, 15). The gltE$^-$ phenotype is difficult to score because these mutants do not show an absolute dependence on streptomycin for growth (14). However, a three-factor cross between strains BB20 and EM111 with *mtl* as the outside marker indicated that the order of these markers is *mtl*, *gltE*, *gpsA* (Table 7 and [by analogy] Fig. 3). The final results of the mapping experiments are given in Fig. 2.

Recombination between gpsA mutants. Bell (1) isolated a number of L-glycerol 3-phosphate dehydrogenase-deficient strains which are probably of independent origin. We have asked whether all these mutants are near to the *gpsA20* allele. *gpsA*$^+$ *str*^{R} recombinants arise very infrequently (when compared to *xyl*$^+$ *metE*$^+$ recombinants) in crosses between various *gpsA*$^-$ strains and CY115 (Table 8). This indicates that the various *gpsA* alleles present in these strains occur very near to the *gpsA20* allele and are probably due to mutations within the same genetic locus defined by *gpsA20*.

Attempts at complementation analysis. To further test if all our mutants deficient in L-glycerol 3-phosphate dehydrogenase possess lesions in the same gene, we attempted genetic complementation tests. We mated strain

TABLE 6. *Order of the gpsA and mtl markers with respect to xyl*[a]

Parents		Distribution of unselected markers		
Donor	Recipient	Classes	No.	Frequency (%)
Cross 1:				
Hfr BB20 (*xyl*$^+$ *mtl*$^+$ *gpsA*$^-$)	X478 (*xyl*$^-$, *mtl*$^-$, *gpsA*$^+$)	*mtl*$^+$ *gpsA*$^+$	104	81
		mtl$^+$ *gpsA*$^-$	9	7
		mtl$^-$ *gpsA*$^+$	15	12
		mtl$^-$ *gpsA*$^-$	0	0
Cross 2:				
Hfr CY119 (*xyl*$^+$ *mtl*$^-$ *gpsA*$^+$)	CY115 (*xyl*$^-$, *mtl*$^+$, *gpsA*$^-$)	*mtl*$^+$ *gpsA*$^+$	0	0
		mtl$^+$ *gpsA*$^-$	0	0
		mtl$^-$ *gpsA*$^+$	96	84
		mtl$^-$ *gpsA*$^-$	18	16

[a] *xyl*$^+$ *str*^{R} recombinants were selected on medium containing L-glycerol 3-phosphate and then scored for unselected markers on the appropriate media.

TABLE 7. *Order of the gpsA and gltE markers with respect to mtl*[a]

Donor	Recipient	Classes	No.	Frequency (%)
Hfr BB20 (*mtl*$^+$ *gltE*$^+$ *gpsA*$^-$)	EM111 (*mtl*$^-$ *gltE*$^-$ *gpsA*$^+$)	*gltE*$^+$ *gpsA*$^+$	42	81
		gltE$^+$ *gpsA*$^-$	6	11
		gltE$^-$ *gpsA*$^+$	4	8
		sltE$^-$ *gpsA*$^-$	0	0

[a] *mtl*$^+$ *tsx*^{R} recombinants were selected on medium containing DL-glycerol 3-phosphate and streptomycin then scored for unselected markers.

CY123 (a *recA*$^-$ derivative of CY115) with strains containing the F′ factors that carry genes in the 70- to 80-min region of the genetic map. Transfer of F140 from a *recA*$^-$ strain to strain CY123 resulted in *gpsA*$^+$ progeny (Table 9). The same donor strain transferring F111 (or F141 or F102 [data not shown]) gave no *gpsA*$^+$ progeny. Thus, F140 carries the *gpsA*$^+$ gene and *gpsA*$^+$ is dominant over *gpsA*$^-$ (a result consistent to that found in vitro [1]). However, F140 is an extremely unstable F′ factor (B. Low, personal communication) in both *rec*$^+$ and *rec*$^-$ strains. Another problem is the frequent occurrence of variants from which parts of the F′ have been deleted (7, 11). Such behavior makes accurate complementation analysis with this F′ extremely difficult and thus it was not attempted.

We have also tried to perform complementation analysis by abortive transduction (2). However, since all the *gpsA* mutants tested formed microscopic colonies on medium lacking DL-glycerol 3-phosphate (which could not be distinguished from the minute colonies formed by abortive transduction), this method could not be used to test for complementation.

TABLE 8. *Recombination between gpsA mutants*

Strain[b]	Recombinants with CY115 (× 10^5)	
	gpsA$^+$ *str*^{R}	*xyl*$^+$ *met*$^+$ *str*^{R}
BB20	<0.001	10.1
BB8	<0.001	4.4
BB17	<0.001	6.4
BB3	<0.001	5.6
BB2	<0.001	7.2
BB11	0.040	2.6
BB28	0.016	3.0
3C3	<0.001	1.0
BB20-14-10d8	<0.001[a]	3.3

[a] This result was obtained when the mating mixture was plated at 42 C. When the plating temperature was 34.5 C, 3.1 × 10^5 *gpsA*$^+$ recombinant colonies were formed. Of 560 of these *gpsA*$^+$ recombinants tested 3 were able to grow at 42 C in the absence of DL-glycerol 3-phosphate.

[b] The genotypes of these strains are the same as that of BB20 (Table 1), except for the *gpsA* allele. None of these strains possesses detectable (<0.3 U/mg of protein) L-glycerol 3-phosphate dehydrogenase activity except strain BB20-14-10d8, which has a temperature-sensitive dehydrogenase (Fig. 1).

TABLE 9. *Complementation with various F′ factors*[a]

Donor strain	Recombinants (× 10^5)			
	gpsA$^+$	*metE*$^+$	*gpsA*$^+$ *metE*$^+$	*xyl*$^+$ *gpsA*$^+$
F111/JC1553	<0.001	0.068	<0.001	
F140/JC1553	10.5			9.8

[a] Strain CY123 was the recipient used. The donors were counter-selected by omission of histidine, leucine, and methionine from the selective medium.

DISCUSSION

The mapping of the *gpsA* locus and the demonstration that this locus is a structural gene for the biosynthetic L-glycerol 3-phosphate dehydrogenase pave the way for isolation of mutants affecting the regulation of the enzyme activity (and hence of phospholipid biosynthesis). However, the sensitive inhibition of this enzyme by its product (10) suggests that control at the level of enzyme synthesis may not be needed. To test this suggestion, we are seeking mutants in which the enzyme is resistant to product inhibition.

Another use of the mapping data is in the construction of *E. coli* strains that carry defects in both the *gpsA* locus and in other loci involved in membrane lipid synthesis. For example, Bell (1) and Kito et al. (9) have reported L-glycerol 3-phosphate auxotrophs of *E. coli* that owe their requirement to a defect in L-glycerol 3-phosphate acyltransferase, the first enzyme in phospholipid biosynthesis. The apparent K_m for L-glycerol 3-phosphate acyltransferase was higher than normal in these mutants. When such acyltransferase K_m mutants are deprived of L-glycerol 3-phosphate, the rate of phospholipid synthesis declines to a few percent of normal (1). This residual synthesis is presumably due to a slow utilization of the L-glycerol 3-phosphate synthesized by the cell. However, a strain possessing a lesion in *gpsA* in addition to the acyltransferase lesion should be completely unable to synthesize phospholipid when starved for L-glycerol 3-phosphate. We have located the acyltransferase K_m mutants at about 68 to 69 min on the linkage map (Cronan and Bell, manuscript in preparation) and thus are in the process of constructing an auxotroph with lesions in both genes.

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