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Genetic Analysis of *Escherichia coli* Mutants Defective in Adenylate Kinase and *sn*-Glycerol 3-Phosphate Acyltransferase

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Complementation analysis with independently isolated *plsA* and *adk* (adenylate kinase) mutants of *Escherichia coli* showed that all the mutants belong to the same complementation group. The results suggest that the *adk* (*plsA*) locus is the structural gene for adenylate kinase.

sn-Glycerol 3-phosphate acyltransferase (EC 2.3.1.15) is an integral membrane enzyme and catalyzes the first committed reaction in phospholipid biosynthesis. A number of mutants of *Escherichia coli* have been isolated that are putatively defective in this enzyme (2, 8, 11). The mutations fall into two classes, *plsA* and *plsB*, and they map at distinct regions of the *E. coli* chromosome (6, 7).

The *plsA* mutants were isolated by a procedure designed to give temperature-sensitive mutants defective in phospholipid biosynthesis (8). They have a more thermolabile *sn*-glycerol 3-phosphate acyltransferase than their parent strains both in vivo and in vitro (7, 8, 10). When the mutants are shifted to the nonpermissive temperature for growth, the rate of phospholipid, DNA, RNA, and protein synthesis decreases in a similar manner (9). This was shown to be due to an increase in the cellular AMP concentration and a drop in the ATP concentration, which in turn results from the inactivation of adenylate kinase (EC 2.7.4.3) (10). Adenylate kinase is responsible for converting AMP to ADP according to the following reaction: AMP + ATP $\rightleftharpoons$ 2 ADP. Thus the inactivation of adenylate kinase causes an increase in the AMP concentration, a decrease in the ADP concentration, and subsequently a drop in the ATP concentration. Regulation of the activity of adenylate kinase may provide the cell with a mechanism for balancing macromolecular synthesis and regulating cell growth (10).

Temperature-sensitive mutants defective in adenylate kinase have been independently isolated (3, 5). When these mutants (here designated *adk*) are shifted to the nonpermissive temperature, the rate of synthesis of RNA, DNA, and protein decreases, and there is a drop in the ATP concentration (4). A comparison of the properties of the *plsA* mutants with the *adk* mutants showed that they have the same phenotype (10). They both show a similar decrease in phospholipid and macromolecular synthesis at the nonpermissive temperature, and they contain a thermolabile adenylate kinase as well as an *sn*-glycerol 3-phosphate acyltransferase that is consistently more thermolabile than the parent strains. To understand the basis for these observations, a complementation analysis was carried out to determine whether the *plsA* mutants fall into the same complementation group as the *adk* mutants or whether they represent a separate genetic locus. Both *plsA* (7) and *akd* (D. Cousin, personal communication) are linked to *purE* at 12 min on the genetic map.

Three independently isolated mutants putatively defective in the *sn*-glycerol 3-phosphate acyltransferase and eight independently isolated mutants putatively defective in adenylate kinase were examined. To eliminate strain difference, all the mutations were transferred into KL218 by transduction with phage P1. All the *plsA* and *adk* mutations cotransduced with *purE*. To prevent recombination between the two alleles that were being tested for complementation, a *recA* mutation (from KL16-99) was placed into the recipient strains by conjugation. These strains were designated KG1, KG2, ... KG11 (Table 1). Two donor strains were constructed for the complementation analysis: one with an episome containing a *plsA* mutation, designated CK1, and the other with an episome containing an *adk* mutation, designated CK2.

Master grids of the two donors, each containing approximately 50 colonies, were grown overnight and printed onto lawns of the recipients. When CK1 and CK2 were the donors, Pro$^+$ His$^+$ strains were selected, and when ORF4/KL251 (*adk*$^+$ *plsA*$^+$) was the donor, Pro$^+$ Trp$^+$ Leu$^+$

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TABLE 1. *Bacterial strains*

Strain	Sex	Genotype[a]	Source
CV2	HfrC	*plsA2 glpD3 glpR2 phoA8 tonA22* T2^r *rel-1* (λ)	J. E. Cronan, Jr.
CV15	?	*plsA15*; other markers as in CV2	J. E. Cronan, Jr.
CV31	HfrC	*plsA31*; other markers as in CV2	J. E. Cronan, Jr.
B121	HfrC	*adk-1 ura glt thi*	D. Cousin
C100	HfrC	*adk-2 ura glt bio pro*	D. Cousin
PA601T28	F′	*adk-28 lac*$^+$ *proC*$^+$ *purE*$^+$*/adk-28 thr leu arg his proA purE thi str*	D. cousin (*adk* mutation from CR341T28)
D100	HfrC	*adk-3*; other markers as in B121	D. Cousin
A13	HfrC	*adk-4*; other markers as in B121	D. Cousin
D135	HfrC	*adk-5*; other markers as in B121	D. Cousin
C108	HfrC	*adk-6*; other markers as in B121	D. Cousin
E112	F$^+$	*adk-7*; other markers as in B121	D. Cousin
KL218	F$^-$	*proC24 purE41 thyA25 nalA12 argG34 metB1 his-53 pyrC30 lac str-97 tsx-63 mlt-2 xyl-7* or *-14*	K. B. Low
KL16-99	Hfr	*thi-1*? *drm-3 recA1 rel-1* (λ$^-$)	K. B. Low
ORF4/KL251	F′	*proC*$^+$ *plsA*$^+$ (*adk*$^+$) *purE*$^+$*/thi-1 metE70 trpE38 purE42 proC32 leu-6 recA1 mtl-1*? *xyl-5 ara-14 lacZ36 azi-6 str-109 tonA23 tsx-67 sup-45* (λ$^-$)	K. B. Low
KG100	F$^-$	*his*$^+$ *recA1*; other markers as in KL218	KL218 × KL16-99
KG1	F$^-$	*plsA2 purE*$^+$ *his*$^+$ *recA1*; other markers as in KL218	See footnote *b*
KG2	F$^-$	*plsA15 purE*$^+$ *his*$^+$ *recA1*; other markers as in KL218	See footnote *b*
KG3	F$^-$	*plsA31 purE*$^+$ *his*$^+$ *recA1*; other markers as in KL218	See footnote *b*
KG4	F$^-$	*adk-1 purE*$^+$ *his*$^+$ *recA1*; other markers as in KL218	See footnote *b*
KG5	F$^-$	*adk-2 purE*$^+$ *his*$^+$ *recA1*; other markers as in KL218	See footnote *b*
KG6	F$^-$	*adk-28 purE*$^+$ *his*$^+$ *recA1*; other markers as in KL218	See footnote *b*
KG7	F$^-$	*adk-3 purE*$^+$ *his*$^+$ *recA1*; other markers as in KL218	See footnote *b*
KG8	F$^-$	*adk-4 purE*$^+$ *his*$^+$ *recA1*; other markers as in KL218	See footnote *b*
KG9	F$^-$	*adk-5 purE*$^+$ *his*$^+$ *recA1*; other markers as in KL218	See footnote *b*
KG10	F$^-$	*adk-6 purE*$^+$ *his*$^+$ *recA1*; other markers as in KL218	See footnote *b*
KG11	F$^-$	*adk-7 purE*$^+$ *his*$^+$ *recA1*; other markers as in KL218	See footnote *b*
CK1	F′	*proC*$^+$ *plsA15 purE*$^+$*/plsA15*	See footnote *c*
CK2	F′	*proC*$^+$ *adk-6 purE*$^+$*/adk-6*	See footnote *c*

[a] For gene symbols, see Bachmann et al. (1).

[b] All the *plsA* and *adk* mutations were transferred into the same genetic background, i.e., KL218, by transduction with phage P1 using the procedure of Lennox (12). Pur$^+$ transductants were selected at 30°C and examined for their temperature sensitivity. The *recA* mutation was introduced into these strains by conjugation with KL16-99. This was carried out at 30°C in L-broth (12). His$^+$ Str^r recombinants were selected and tested for the presence of *recA* by the rate of colony formation on L-broth plates containing 0.5 μg of mitomycin C per ml (13).

[c] F′ factor ORF4 was introduced into the two transductants of KL218 that contained either the *plsA15* mutation from CV15 or the *adk-6* mutation from E112 (the construction of these two transductants is described in footnote b). This was done by mating and selecting for merodiploid strains that were Pro$^+$ Trp$^+$ Leu$^+$. These strains were temperature resistant, indicating that the *plsA*$^+$ (*adk*$^+$) episome was dominant. Rare spontaneous homogenates were isolated from these strains that were temperature sensitive and acted as donors at high frequency (*plsA/plsA* or *adk/adk*).

TABLE 2. *Complementation of plsA and adk mutants*[a]

Recipient	CK1 donor			CK2 donor			ORF4/KL251 donor		
	Growth at 30°C	Growth at 40°C	Presence of *recA*	Growth at 30°C	Growth at 40°C	Presence of *recA*	Growth at 30°C	Growth at 40°C	Presence of *recA*
KG1	+	−	+	+	−	+	+	+	+
KG2	+	−	+	+	−	+	+	+	+
KG3	+	−	+	+	−	+	+	+	+
KG4	+	−	+	+	−	+	+	+	+
KG5	+	−	+	+	−	+	+	+	+
KG6	+	−	+	+	−	+	+	+	+
KG7	+	−	+	+	−	+	+	+	+
KG8	+	−	+	+	−	+	+	+	+
KG9	+	−	+	+	−	+	+	+	+
KG10	+	−	+	+	−	+	+	+	+
KG11	+	−	+	+	−	+	+	+	+
KG100	+	+	+	+	+	+	+	+	+

[a] Complementation was carried out by print-matings essentially as described by Low (14). A grid of donor colonies was grown overnight on plates containing medium selective for maintenance of the episome [0.4 g of sodium citrate, 0.1 g of $MgSO_4 \cdot 7H_2O$, 1.0 g of $(NH_4)_2SO_4$, 2.0 g of KH_2PO_4, 7.0 g of K_2HPO_4, 4.0 g of glucose, 15 g of agar, 40 mg of any required amino acid or nucleotide, and 1 mg of filtered thiamine hydrochloride per liter] and replica-plated onto two plates containing lawns of the recipients spread on selective plates. The plates were incubated for 1 h at 30°C, and then one of the plates was transferred to the nonpermissive temperature to determine the temperature-sensitive phenotype of the F-ductants. Additional plates were made to insure that the donors and recipients had not reverted. The F-ductants that grew on the 30°C plate were picked and tested for growth at 40°C and for the presence of *recA*. The results of growth at 40°C from the F-ductants picked at 30°C gave the same results as the direct incubation at 40°C, and only one column is shown for these results.

strains were selected. The F-ductants made from all the *plsA* and *adk* strains grew at the permissive temperature but not at the nonpermissive temperature, indicating that all the *plsA* and *adk* mutants belonged to the same complementation group (Table 2). The colonies that grew at the permissive temperature were further examined at the nonpermissive temperature and for the presence of *recA* to insure the validity of the results. Control matings with either donor or recipient strains that were *adk*$^+$ (*plsA*$^+$) showed good growth at the nonpermissive temperature. Pur$^+$ Pro$^+$ His$^+$ cells were selected when KG100 was used as the recipient. The episome markers, *proC*$^+$ and *purE*$^+$, are on either side of *adk* (*plsA*), and selection for both markers further indicates that the episome was transferred properly.

The complementation results would not be valid if the *adk* (*plsA*) episomes in CK1 and CK2 contained a deletion involving more than one gene. To eliminate this possibility, temperature-resistant *adk*$^+$ (*plsA*$^+$) revertants were obtained. The revision frequencies for CK1 and CK2 were 3×10^{-8} (40.0°C) and 2×10^{-7} (40.9°C) reversions per bacterium per generation, respectively, as determined by the method of Luria and Delbrück (15). This is similar to the reversion frequencies for KG1, KG2, ... KG11, which fell in the range 10^{-6} to 4×10^{-9} reversions per generation per bacterium at 40.0 to 40.9°C (data not shown). These reversion frequencies are consistent with a single point mutation. To determine whether the *adk* (*plsA*) gene on the episome or the chromosome had reverted in the temperature-resistant colonies of CK1 and CK2, the episomes were transferred into an *akd* (*plsA*) *recA* recipient. A large number of these F-ductants were temperature resistant (data not shown), which indicates that the original episomes of CK1 and CK2 must have contained point mutations.

Adenylate kinase has been purified from a temperature-sensitive *adk* mutant (5, 21) (strain CR341T28, whose *adk* mutation is the same one as in KG6). Although the thermolability of the enzyme isolated from the mutant strain changes during purification, the enzyme itself appears to be thermolabile. As shown in this study, all the *plsA* and *adk* mutations belong to the same complementation group, and consequently this locus appears to be the structural gene for adenylate kinase. The inactivation of adenylate kinase at temperatures nonpermissive for growth can account for the observed phenotype of the *adk* (*plsA*) mutants (10). There appears to be a block in initiation of gene transcription which occurs slightly before bulk protein synthesis decreases (16). Snider and Kennedy (20) solubilized and partially purified the *sn*-glycerol 3-phosphate acyltransferase from a *plsA* mutant that was temperature sensitive and from a *plsB* mutant with a higher K_m for *sn*-glycerol 3-phosphate when assayed in membrane preparations. The partially purified enzyme from the *plsA* mutant was not thermolabile, whereas the en-

zyme from the *plsB* mutant had a higher K_m as compared with the wild-type enzyme. Thus, their data supported the view that *plsB* is a structural gene for the *sn*-glycerol 3-phosphate acyltransferase and not *plsA*. In addition, V.A. Lightner (Fed. Proc. **38:** 471, 1979) and Snider (19) have shown that strains with a hybrid plasmid containing the *plsB* gene have a large increase in the specific activity of the *sn*-glycerol 3-phosphate acyltransferase.

On the other hand, there is a considerable amount of evidence to show that the *sn*-glycerol 3-phosphate acyltransferase is altered in the *adk* (*plsA*) mutant class (for a discussion see reference 10). Kito et al. have isolated another mutant that contains an *sn*-glycerol 3-phosphate acyltransferase with an altered K_m for glycerol 3-phosphate (11). Adenylate kinase has not been studied, and the mutation has not been mapped. However, this mutant behaves similarly to *adk* (*plsA*) mutants. In the absence of glycerol 3-phosphate, there is a reduced rate of phospholipid synthesis and cell growth, which has been ascribed to lower ATP levels (17). Recently Ray et al. have shown that when *plsA* mutants are shifted to the lower end of the nonpermissive temperature range (approximately 35°C, where there is some growth), phospholipid synthesis was inhibited, whereas nucleic acid synthesis was inhibited only slightly (18). This appeared to be caused by the thermolability of the *sn*-glycerol 3-phosphate acyltransferase. Although the data suggesting that *sn*-glycerol 3-phosphate acyltransferase is altered in *adk* (*plsA*) mutants can be rationalized in different ways, one possible explanation for the available data is that adenylate kinase and the *sn*-glycerol 3-phosphate acyltransferase interact or exist in a complex (10). This would explain how one mutation (in adenylate kinase) could also alter the properties of the *sn*-glycerol 3-phosphate acyltransferase. This complex may not be strong and could be disrupted by breaking the cells or by using detergents. An association of the two enzymes would provide the cell a means of linking membrane synthesis with the rest of cellular metabolism, and it could be one of the factors in promoting balanced cell growth.

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