Escherichia coli Mutants with Temperature-sensitive Phosphatidylserine Synthetase: Genetic Analysis

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Bacterial mutants with modifications in membrane lipids are potentially valuable in the study to elucidate the roles of lipids in membrane and cell physiology. We isolated a number of mutants of *Escherichia coli* K-12 with temperature-sensitive formation of phosphatidylethanolamine (PE).^{1,2)} The biochemical defect of one of the mutants, strain OS294, was identified to be phosphatidylserine (PS) synthetase [EC 2.7.8.8], the enzyme catalyzing the synthesis of the immediate precursor to PE.³⁾ The gene *pss* (PS synthetase) which is responsible for the temperature-sensitivity of the strain OS294 has now been mapped on the chromosome at 50 min according to the designation by Taylor and Trotter.⁴⁾

Since the strain OS294 inherited Hfr property from its parent strain CS101 (HfrC, *metB*), the temperature-sensitivity of OS294 was first transferred to an F⁻ strain, JE1011 (*TL*, *trp*, *his*, *thy*, *gal*, *str*^R, Y. Sugino strain), by conjugation at 30°C, at which PE formation in OS294 cells was not strongly inhibited. Using a resultant F⁻*pss*⁻*gal*⁺*met*⁺ recombinant and several Hfr strains with different points of origin, a replica mating experiment was carried out. The result revealed that the *pss* locus was positioned between the points of origins of strain KL16 and KL983 (45~55 min on the *E. coli* chromosome map⁴⁾). More detailed analysis was done through an interrupted mating experiment between strain KL16 and strain OS1420 (see legend for Fig. 1). *Pss*⁺ and *tyrA*⁺ were transferred

FIG. 1. Localization of *pss* by Interrupted Mating.

An Hfr strain KL16 (transferring clockwise from 55 min, K. B. Low strain) and an F⁻ strain OS1420 (*pss*, *his*, *tyrA*, *thy*, *str*^R) were grown in NBY medium²⁾ at 37°C and 27°C, respectively. Log-phase cells of both cultures were mixed at zero time in a ratio of 1:6 (total 2.8×10^8 cells/ml), and incubated at 32°C with gentle aeration. Aliquots (0.2 ml) were withdrawn at intervals into ice-chilled saline (1.8 ml) and immediately vortexed for 2 min on a Thermomixer. Plating was done at various degrees of dilution onto appropriate selective media. *Pss*⁺ recombinants were scored on NBY agar (NBY with 1.5% agar) after 42°C incubation. Strain OS1420 was constructed by conjugation of an HfrC *pss*⁻ *gal*⁺ *met*⁺ derivative of JE1011 and a strain carrying *tyrA*⁻, derived from KL262 (K. B. Low strain).

■—■, *thy*⁺ *str*^R; ○—○, *tyrA*⁺ *str*^R; ●—●, *pss*⁺ *str*^R; □—□, *his*⁺ *str*^R.

almost at the same time as shown in Fig. 1. It was therefore presumed that the *pss* gene was located very close to the *tyrA* locus.

The results of the conjugation experiments led us to analyze the co-transduction by phage P1vir of *pss* with *tyrA* and its adjacent markers. *TyrA* was best co-transducible with *pss* at the frequency of 41~62%. *PheA*, *nadB*, *nadR* and *purI* were also co-transduced with *pss* at 44, 30, 21 and 24% of frequency, respectively. We examined further the pre-

TABLE I. THREE-FACTOR ANALYSIS OF THE GENES LOCATED IN THE *pss* REGION

Transductions were performed by P1-mediated genetic transfer following the usual basic procedures.⁶⁾ P1-donor strain, OS2020 (F^- , *pss*, *thy*, *str^R*), was constructed by four-step recombinations from OS294, JE1011, KL16 and KL262 (K. B. Low strain). Recipients, UTH7033 (*purI*, *tyrA*) and UTH4150 (*purI*, *nadB*) were from G. J. Tritz.

Cross No.	Transduction	Genotype			Total No. of each genotype	
		Selected marker	Unselected marker			
1	OS2020 × UTH7033	<i>tyrA</i> ⁺	<i>pss</i> [−]	<i>purI</i> ⁺	29	
		<i>tyrA</i> ⁺	<i>pss</i> [−]	<i>purI</i> [−]	37	
		<i>tyrA</i> ⁺	<i>pss</i> ⁺	<i>purI</i> ⁺	1	
		<i>tyrA</i> ⁺	<i>pss</i> ⁺	<i>purI</i> [−]	92	
2 ^{a)}	OS2020 × UTH7033	<i>purI</i> ⁺	<i>pss</i> [−]	<i>tyrA</i> ⁺	13	10
		<i>purI</i> ⁺	<i>pss</i> [−]	<i>tyrA</i> [−]	2	5
		<i>purI</i> ⁺	<i>pss</i> ⁺	<i>tyrA</i> ⁺	1	2
		<i>purI</i> ⁺	<i>pss</i> ⁺	<i>tyrA</i> [−]	184	92
3	OS2020 × UTH4150	<i>purI</i> ⁺	<i>pss</i> [−]	<i>nadB</i> ⁺	45	
		<i>purI</i> ⁺	<i>pss</i> [−]	<i>nadB</i> [−]	1	
		<i>purI</i> ⁺	<i>pss</i> ⁺	<i>nadB</i> ⁺	16	
		<i>purI</i> ⁺	<i>pss</i> ⁺	<i>nadB</i> [−]	128	

^{a)} Cross 2 was repeated under the identical conditions.

cise positional relationship between *pss* and other markers by three-factor cross experiments. In all three crosses shown in Table I, a *pss*⁻ strain, OS2020 was used as P1 donor and double auxotrophic mutants (*purI*⁻*tyrA*⁻ and *purI*⁻*nadB*⁻) were used as recipients. If the gene order in this region was *pss-purI-tyrA*, the genotype with the lowest frequency among *tyrA*⁺ recombinants of Cross 1 should be *tyrA*⁺*pss*⁻*purI*⁻, because of the necessity of quadruple crossing-over within the donor and recipient DNAs. On the other hand, if the gene order was *purI-tyrA-pss*, the lowest of four genotypes in Cross 2 would be *purI*⁺*pss*⁻*tyrA*⁻ as the result of quadruple crossing-over. However, the actual genotype with the lowest frequency was *tyrA*⁺*pss*⁺*purI*⁺ both in Crosses 1 and 2, and this result was only consistent with the gene order of *purI-pss-tyrA*. The relation of *pss* and *nadB*, the gene being also positioned between *purI* and *tyrA*,⁶⁾ was then examined by Cross 3. The genotype with the lowest frequency in this case was *purI*⁺*pss*⁻*nadB*⁻. We therefore concluded that the gene order in *pss* region is *purI-nadB-pss-tyrA*.

The temperature-sensitive growth and PE formation, *in vivo*, and PS synthetase defect, *in vitro*, in *pss*⁻ strains, constructed by co-transduction of *pss*⁻ with *tyrA*⁺ into fresh genetic background, were also demonstrated under almost the same assay conditions as employed previously,³⁾ and some temperature-resistant revertants isolated from one of the *pss*⁻ transductants (reversion frequency: 5×10^{-9} /cell) regained the enzyme activity both at 27°C and 42°C. Therefore the *pss* locus and the defect in PS synthetase activity acted as a single genetic entity. By modifying the extraction and assay conditions, we were able to detect PS synthetase in a *pss*⁻ strain which otherwise was abolished completely upon disruption of the cells.³⁾ This activity decreased quickly by pretreatment at 42°C. This strongly suggests that *pss* is the structural gene for PS synthetase.

The locus of *pss* demonstrated here is far from the gene (12 min) described by Cronan⁷⁾ to be responsible for the temperature-sensitive formation of PE in his mutant strain C4, on which biochemical characterization has not been reported.

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