The *thiJ* locus and its relation to phosphorylation of hydroxymethylpyrimidine in *Escherichia coli*

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Extracts from *Escherichia coli* K-12 contained two distinct enzymes capable of catalysing the phosphorylation of hydroxymethylpyrimidine (HMP) to HMP monophosphate: pyridoxine kinase (EC 2.7.1.35) and an enzyme that has not previously been genetically analysed, HMP kinase (EC 2.7.1.49). Two distinct genes, *pdxL* and *thiJ*, specify the activities of the former and latter enzymes, respectively. The inactivation of both genes by independent mutations in the same cell resulted in the complete loss of HMP kinase activity. Experiments with a series of strains that carry mutations in *thiC*, *thiC pdxB*, *thiC pdxB pdxL* and *thiC pdxB pdxL thiJ* revealed that the ability of the double mutant (*pdxL thiJ*) to utilize HMP in thiamin pyrophosphate biosynthesis was restored by introducing the wild-type allele corresponding to the *thiJ* mutation. The *thiJ* locus was mapped on the chromosome near the *thiD* and *thiM* loci, which govern the activities of phosphomethylpyrimidine kinase (EC 2.7.4.7) and hydroxyethylthiazole kinase (EC 2.7.1.50), respectively.

Keywords: *Escherichia coli*, thiamin biosynthesis, *thiJ* locus, hydroxymethylpyrimidine

INTRODUCTION

In *Escherichia coli* five enzymes are involved in the biosynthesis of thiamin pyrophosphate (thiamin-PP) from 2-methyl-4-amino-5-hydroxymethylpyrimidine (hydroxymethylpyrimidine, HMP) and 4-methyl-5-(β -hydroxyethyl)thiazole (hydroxyethylthiazole, HET) via the intermediate formation of thiamin monophosphate (thiamin-P) (Nakayama & Hayashi, 1972a). Another enzyme catalyses the formation of thiamin-P from thiamin (Nakayama & Hayashi, 1972b). The genes specifying five of these six enzymes, *thiB*, *thiD*, *thiK*, *thiL* and *thiM*, are distributed on the chromosome in four distinct regions (Kawasaki *et al.* 1968; Mizote & Nakayama, 1989b) (Fig. 1). Among these, *thiD* and *thiM*, which code for phosphomethylpyrimidine (hydroxymethylpyrimidine monophosphate, HMP-P) kinase (EC 2.7.4.7) and HET kinase (EC 2.7.1.50) activities, respectively, are located

close together on the chromosome at about 46 min (Mizote & Nakayama, 1989b). However, genetic analysis of HMP kinase (EC 2.7.1.49), which catalyses the phosphorylation of HMP to HMP-P, has not been possible because strains deficient in this enzyme have never been reported. We previously characterized an

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Abbreviations: HMP (hydroxymethylpyrimidine), 2-methyl-4-amino-5-hydroxymethylpyrimidine; HMP-P, HMP monophosphate; HMP-PP, HMP pyrophosphate; HET (hydroxyethylthiazole), 4-methyl-5-(β -hydroxyethyl)-thiazole; thiamin-P, thiamin monophosphate; thiamin-PP, thiamin pyrophosphate; POL, pyridoxol; POL-P, pyridoxol 5-phosphate; PAL, pyridoxal; PAL-P, pyridoxal 5-phosphate; PAM, pyridoxamine.

Fig. 1. Biosynthetic pathway for thiamin-PP from HMP and HET in *E. coli*. Parentheses indicate approximate map positions (min) of each of the genes.

HMP-phosphorylating enzyme purified from *E. coli* K-12 cells grown in minimal medium and showed that the enzyme phosphorylates each of the pyridoxine substrates, pyridoxol (POL), pyridoxal (PAL) and pyridoxamine (PAM) as well as HMP and that the phosphorylation of HMP is competitively inhibited by POL and vice versa. This led to the conclusion that HMP is phosphorylated by the enzyme previously designated pyridoxine kinase (PN/PL/PM kinase; pyridoxal kinase; EC 2.7.1.35), (Mizote & Nakayama, 1989a).

Subsequently, we succeeded in isolating strains that showed a reduced kinase activity for both HMP and POL by a second mutation in a strain already carrying the *pdxB* mutation. For the second mutated gene we tentatively gave the designation *thiN* (Nakayama, 1990). However, our present investigation has revealed that pyridoxine kinase is lacking in this class of strains, even though extracts from the strain possess lower HMP kinase activity than the parent strain. This reduction in activity has no effect on the utilization of HMP for thiamin-PP biosynthesis in the living cell and led us to change the designation of this gene from *thiN* to *pdxL*. Additional evidence in support of this redesignation was obtained from experiments with a strain totally defective in HMP kinase. The locus of the new mutation, for which the symbol *thiJ* will be used here, was situated in a region near the *thiD* and *thiM* loci.

METHODS

***E. coli* strains.** The bacterial strains constructed for this study are listed in Table 1. The principal strains used in the experiments are all derived from a polyauxotrophic strain,

JC1552. The biosynthesis of thiamin-PP in this strain from HMP and HET has been described previously (Imamura & Nakayama, 1982).

Media and growth conditions. Minimal medium 56 as modified by Low (1973) was used for the growth experiments including the selection for the desired strains. Mutants reported here were produced by treatment of parent cells with N-methyl-N'-nitro-N-nitrosoguanidine (NTG) by the method reported by Adelberg *et al.* (1965). In some experiments ethylmethane sulfonate (EMS) was used, following the method of Osborn *et al.* (1967). Mating and strain constructions were performed by procedures essentially as described by Low (1973) and Miller (1972). Phage P1 *kc*-mediated transductions were performed according to Lennox (1955) and Taylor & Trotter (1967). Luria broth was used in transduction experiments, and Luria broth without glucose was used in conjugation experiments.

Enzyme assay. Enzyme activity with crude extracts and with whole cells were measured as described previously (Nakayama & Hayashi, 1972b). The amounts of HMP-P and hydroxymethylpyrimidine pyrophosphate (HMP-PP) formed were estimated by microbiological assay with paper bioautography using *E. coli* strain 70-17. Detailed assay conditions with this strain have been described previously (Nakayama & Hayashi, 1979). The amount of phosphate esters of the pyridoxine substrates formed was assayed with *E. coli* strain H5-619, a strain auxotrophic for pyridoxine, by essentially the same procedure as that for HMP-P.

Chemicals. HMP, 2-methylthio-4-amino-5-hydroxymethylpyrimidine (methylthiohydroxymethylpyrimidine) and 2-methyl-4-amino-5-bromomethylpyrimidine dihydrobromide (bromomethylpyrimidine) were gifts from Takeda Chemical Industries, Osaka, Japan. HMP-P and HMP-PP were prepared from bromomethylpyrimidine according to the method of Lewin & Brown (1961). POL, PAL, PAM, 4-deoxypyridoxine and pyridoxal 5-phosphate (PAL-P) were purchased from

Table 1. *E. coli* strains

Strains	Relevant genotype*	Relevant phenotype†	Source
JC1552	<i>argG6 metB1 his-1 leu-6 trp-31 rpsL104</i>		CGSC‡
SN372	JC1552 <i>thiC21</i>	Hmp ⁻	Isolated from JC1552 by mutagenesis
SN372L	SN372 <i>leu</i> ⁺	Hmp ⁻	SN372 × BW113
SN372L-3	SN372L <i>pdxB20</i>	Hmp ⁻ Pol ⁻	Isolated from SN372L by mutagenesis
SN372L-3-9	SN372L-3 <i>pdxL2</i>	Hmp ⁻ Pal ⁻	Isolated from SN372L-3 by mutagenesis
SN372L-3-9-10	SN372L-3-9 <i>thiJ1</i>	Thi ⁻ Pal ⁻	Isolated from SN372L-3-9 by mutagenesis
M-3-9-6	SN372L-3-9-10 <i>thiJ</i> ⁺ <i>thiM1</i>	Hmp ⁻ Pal ⁻	SN372L-3-9-10 × P1 _{kc} (SN164L-1)
K3-9-101	SN372L-3-9-10 <i>pdxL</i> ⁺ <i>nupC3146::Tn10kan</i>	Thi ⁻ Pol ⁻	SN372L-3-9-10 × P1 _{kc} (CAG18565)
SN372L-3-T1	SN372L-3 <i>pdxL2 nupC3146::Tn10kan</i>	Hmp ⁻ Pol ⁻	SN372L-3 × P1 _{kc} (SNK372L-3-9-10)
SN164L-1	JC1552 <i>leu</i> ⁺ <i>thiA7 thiM1</i>	Thi ⁻	Mizote & Nakayama (1989b)
AB2557	<i>purF1 aroC4 dsdA1</i>		CGSC‡
AB2557F	AB2557 <i>purF</i> ⁺ <i>pdxB20</i>	Pol ⁻	AB2557 × P1 _{kc} (SN372L-3)
CAG18565	<i>nupC3146::Tn10kan</i>		NIG§

* Genetic nomenclature is as given by Bachmann (1983).

† Hmp⁻, strains requiring either thiamin or HMP for growth; Thi⁻, strains requiring thiamin for growth, and incapable of growth on either HMP or HET; Pol⁻, strains requiring POL or PAL at concentrations above 0.1 µM for maximum growth; Pal⁻, strains capable of growth on PAL to the same extent as Pol⁻ strains, but incapable of growth on POL at concentrations below 0.1 µM and requiring POL at concentrations above 10 µM for maximum growth.

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Sigma. Pyridoxol 5-phosphate (POL-P) was a gift from Dr A. Iwashima.

RESULTS AND DISCUSSION

Isolation and characterization of a *pdxL* mutant

Our previous study (Nakayama, 1990) showed that strains with reduced HMP kinase activity can be isolated from a *pdxB* strain by selection for deoxypyridoxine resistance. All of the resistant strains isolated showed the same growth characteristics (Pol⁻) as described in the footnote of Table 1. To determine whether or not the strains of this class can utilize HMP for thiamin-PP biosynthesis, a *thiC* strain which requires HMP for growth was initially produced, followed by the isolation of a series of strains with additional mutations in the loci of *pdxB*, *pdxL* and *thiJ*.

The *thiC* (Ts) *pdxB* (Ts) strain (SN372L-3) was mutagenized with EMS and cultured in minimal medium containing 20 nM thiamin and 0.1 µM PAL at 37 °C for 24 h. The culture was washed with saline and a portion was mixed in minimal agar to yield approximately 10⁹ cells per plate. A paper disk containing 10 nmol deoxypyridoxine was placed on the centre of the plate and the plate was incubated at 25 °C for 72 h. Resistant colonies growing near the paper were isolated at random and purified. Mutants that could grow on 0.2 µM PAL but not on 0.2 µM POL at 37 °C were selected using medium containing thiamin by the replica-plating method of Lederberg & Lederberg (1952). It has been shown that POL-P oxidase can catalyse the conversion from POL-P into PAL-P in *E. coli* (Turner & Happold, 1961; Henderson, 1965) and that the *pdxH* mutation, which is responsible for the loss of the oxidase activity and for the growth requirement of PAL, is located at 36 min on the genetic map (Shimizu & Dempsey, 1976; Lam & Winkler, 1992). Our *pdxL* mutants differ from *pdxH* mutants in their enzymic deficiencies, POL requirements and genetic map positions.

Location of *pdxL*

Preliminary matings placed the site of the *pdxL* gene between *supN* and *dsdA* on the chromosome. In these matings and transductional crosses, in which we wished to use *pdxL* as an unselected marker, it was necessary to use a recipient strain in which the *de novo* synthetic pathway for POL-P had been blocked by a mutation such as *pdxB* that also carried several selectable mutations in the region from 49 to 52 min. For this purpose strain AB2557F (*dsdA aroC pdxB*) was infected with P1 grown on strain SN372L-3-9 and the transductants formed were first selected for the inheritance of both *dsdA*⁺ and *aroC*⁺ simultaneously on plates containing PAL, followed by scoring the number of those transductants which had also acquired *pdxL*. Among 45 transductants, only 2 required PAL for growth. This suggested that *pdxL* is situated at about 1.0 min clockwise from *aroC*. This was further supported by an experiment in which transduction was performed using strain CAG18565 (*nupC3146::Tn10kan*)

Fig. 2. Formation of HMP-P from HMP by extracts prepared from *E. coli* SN372L-3-9 cells grown in minimal media containing increasing concentrations of thiamin plus 100 nM PAL (a) or increasing concentrations of PAL plus 3 nM thiamin (b). The reactions and assay systems are described in Methods. Results shown are the means $\pm$ SD of three different experiments.

as donor and strain SN372L-3-9 as recipient. In this cross, if *nupC* and *pdxL* are located very close together, Km^r *pdxL*⁺ *pdxB* transductants would appear at high frequencies. The results showed that 36 of 42 Km^r transductants had gained the ability to grow on POL in the presence of HMP, whereas the remaining transductants still required PAL under the same conditions. On the other hand, when strain SN372L-3-9 was replaced by strain SN372L-3-9-10 in the same cross and the cotransduction of Km^r with *pdxL*⁺ was examined, 23 of 27 Km^r transductants had gained the ability to grow on POL in the presence of thiamin. These results indicated that the *pdxL* gene is situated very close to *nupC*, probably within less than 0.1 min.

Isolation and characterization of *thiJ* mutant

The *thiC* (Ts) *pdxB* (Ts) *pdxL* mutant (SN372L-3-9) was mutagenized with NTG and cultured in minimal medium containing 1 mM glycolaldehyde at 25 °C for 40 h. Methylthiohydroxymethylpyrimidine-resistant mutants were selected by a method essentially the same as that used in the selection for deoxypyridoxine resistance. In this case a paper disk which contained 0.1 µmol of this HMP analogue was placed on minimal agar plate supplemented with 1 mM glycolaldehyde. Mutants that could grow on 20 nM thiamin but not on the same concentration of HMP at 37 °C were then selected on minimal medium containing glycolaldehyde. One such mutant, strain SN372L-3-9-10, was used for further studies.

Preliminary matings placed the site of the *thiJ* gene near 46 min, presumably close to a region where two thiamin genes, *thiD* and *thiM*, are located (Imamura & Nakayama, 1981; Mizote & Nakayama, 1989b). In this report, we determined cotransduction between *thiJ* and *thiM* with

Table 2. Phosphorylation of HMP, POL and PAL by extracts from mutant cells grown at different concentrations of thiamin

Extracts prepared from cells grown on the indicated concentrations of thiamin plus 100 nM PAL were incubated with each of the three substrates, HMP, POL and PAL in the reaction mixture. The amount of the corresponding phosphate ester formed was then measured. Results are given in nmol phosphate ester formed (mg protein)⁻¹ h⁻¹ and are the means $\pm$ SD of three different experiments.

Strain (Relevant genotype)*	Phosphate ester formed	Thiamin concn (nm)		
		3	20	100
SN372L-3 (<i>pdxL</i> ⁺ <i>thiJ</i> ⁺)	HMP-P	7.82 $\pm$ 0.44	8.94 $\pm$ 0.29	8.81 $\pm$ 0.33
	POL-P	1.05 $\pm$ 0.06	1.12 $\pm$ 0.10	1.18 $\pm$ 0.09
	PAL-P	1.87 $\pm$ 0.22	1.35 $\pm$ 0.13	1.21 $\pm$ 0.14
SN372L-3-9 (<i>pdxL</i> <i>thiJ</i> ⁺)	HMP-P	6.00 $\pm$ 0.33	0.33 $\pm$ 0.04	< 0.01
	POL-P	0.21 $\pm$ 0.03	0.20 $\pm$ 0.04	< 0.01
	PAL-P	0.97 $\pm$ 0.09	0.80 $\pm$ 0.12	1.01 $\pm$ 0.11
SN372L-3-9-10 (<i>pdxL</i> <i>thiJ</i>)	HMP-P	< 0.01	< 0.01	< 0.01
	POL-P	< 0.01	< 0.01	< 0.01
	PAL-P	0.17 $\pm$ 0.04	0.22 $\pm$ 0.04	0.19 $\pm$ 0.04

* Plus *thiC* *pdxB* for all strains.

strain SN164L-1 (*thiA thiM*) as donor and strain SN372L-3-9-10 as recipient. Since in the recipient strain *thiC*, *pdxB* and *pdxL* mutations are all further away from *thiM* than the maximal length of the chromosomal fragment carried by a single phage particle, three types of transductants with respect to the *thiM* mutation, *thiJ thiM*, *thiJ*⁺ *thiM* and *thiJ*⁺ *thiM*⁺, would appear in this cross. Transductants that had received *thiJ*⁺ could be selected for their ability to grow on a combination of PAL and HMP. If the *thiJ* and *thiM* genes are situated close together, the unselected donor trait *thiM* would be cotransduced with *thiJ*⁺ at high frequencies. To determine whether or not the transductants had received the *thiM* mutation, HET kinase activity was assayed in cell extracts by the procedure described previously (Mizote & Nakayama, 1989b). The results showed that 31 of 39 *thiJ*⁺ transductants possessed only about 1/20 to 1/40 the HET kinase activity of strain SN372L-3-9-10, while in the remaining 8 *thiJ*⁺ transductants the kinase activities were comparable to those of the recipient, showing that the *thiJ* mutation is 78–80% linked to the *thiM* allele.

Phosphorylation of HMP, POL and PAL by extracts of mutant cells

To investigate whether or not a regulatory mechanism is involved in the synthesis of the *thiJ*-encoded enzyme, mutant cells were grown in two different series of minimal media, one containing increasing concentrations of thiamin and 100 nM PAL and the other containing increasing concentrations of PAL and 3 nM of thiamin. The data obtained clearly showed that increasing the concentration of thiamin in the growth medium resulted in decreasing levels of HMP kinase activity (Fig. 2a). The concentration of thiamin that caused full repression of

enzyme activity was above 100 nM, full derepression being evident at thiamin concentrations below 3 nM. Contrasting results were obtained with PAL; in this case the activity remained at a constant level at all concentrations (Fig. 2b).

Subsequently, the phosphorylation of HMP, POL and PAL were assayed with extracts from mutant cells grown in minimal medium supplemented with 100 nM PAL together with thiamin at final concentrations of 3, 20 and 100 nM (Table 2). In strain SN372L-3 (*pdxL*⁺ *thiJ*⁺) the amount of the phosphate esters formed from the respective substrates appeared to be almost independent of the concentration of thiamin added to minimal medium, though the extracts showed the kinase activity for HMP to be much higher than either for POL or for PAL. In strain SN372L-3-9 (*pdxL* *thiJ*⁺) neither HMP-P nor POL-P was formed by extracts from cells grown on thiamin at 100 nM. Although decreasing the concentration of thiamin in the growth medium resulted in increased levels of HMP kinase activity, only a small amount of POL-P formation could be detected under the same conditions. With regard to the phosphorylation of PAL, the extracts showed approximately 60–80% as much activity as did extracts from strain SN372L-3. The addition of thiamin to the medium did not have any effect on the kinase activity toward this substrate. In strain SN372L-3-9-10 (*pdxL* *thiJ*), neither HMP-P nor POL-P was formed by extracts from cells grown on thiamin even at a concentration as low as 3 nM, at which thiamin limited the growth yield. Contrary to expectation, the formation of PAL-P could still be demonstrated, though the activity was only 20% of that of strain SN372L-3-9. The most reasonable explanation of these results is that two distinct enzymes are involved in the phosphorylation of HMP: an enzyme

Table 3. Formation of HMP-P and HMP-PP from HMP by cell suspensions of mutant strains

Cells grown on 100 nM PAL and 3 nM thiamin were incubated with HMP in the reaction mixture. The amounts of HMP, HMP-P and HMP-PP in the cells were then measured. Results are given in nmol HMP phosphate formed (mg dry wt cells)⁻¹ h⁻¹ and are the means $\pm$ SD of three different experiments.

Strain	Relevant genotype*	HMP-P	HMP-PP
SN372L-3	<i>pdxL</i> ⁺ <i>thiJ</i> ⁺	1.21 $\pm$ 0.10	0.55 $\pm$ 0.09
SN372L-3-9	<i>pdxL thiJ</i> ⁺	1.05 $\pm$ 0.11	0.14 $\pm$ 0.02
SN372L-3-9-10	<i>pdxL thiJ</i>	< 0.01	< 0.01
M3-9-6	<i>pdxL thiJ</i> ⁺	3.33 $\pm$ 0.14	8.75 $\pm$ 0.34
K3-9-101	<i>pdxL</i> ⁺ <i>thiJ</i>	0.43 $\pm$ 0.09	< 0.01

* Plus *thiC pdxB* for all strains.

previously designated pyridoxine kinase (PdxL kinase) and an enzyme that has not previously been genetically analysed, now referred to as an HMP kinase (ThiJ kinase). The activity of the *thiJ*-encoded kinase can be measured only in strains with a defective pyridoxine kinase grown with a limited availability of thiamin. Under these conditions the expression of the *thiJ* gene is increased. The present results also suggest that, apart from the two kinases, no other enzymes catalyse the phosphorylation of HMP. The existence of a third enzyme that might catalyse the formation of PAL-P from PAL more efficiently than that of POL-P from POL is suggested since strain SN372L-3-9-10 was capable of catalysing the PAL phosphorylation reaction at an appreciable level and growth was still supported by thiamin plus PAL.

Physiological effects of the *thiJ* and the *pdxL* mutations

To obtain a more complete understanding of the physiological role of the *pdxL* and *thiJ* genes in the biosynthesis of thiamin-PP, the *pdxL* mutation of strain SN372L-3-9-10 was transduced into strain SN372L-3 and the transductants were tested for their ability to phosphorylate HMP. Extracts from 10 of the transductants (SN372L-3-T1 to SN372L-3-T10) produced 6.20 ± 0.32 nmol HMP-P under the standard assay conditions when they were grown on a limited amount of thiamin, whereas no appreciable kinase activity could be detected when the transductants were grown in the presence of excess thiamin. The introduction of the *pdxL* mutation into strain SN372L-3 therefore resulted in a mutant having the same characteristics as strain SN372L-3-9 with respect to the phosphorylation of HMP.

When *E. coli* cell suspensions were incubated under aerobic conditions with HMP in the presence of glucose, HMP was taken up into the cell and HMP-PP was formed in large amounts via the intermediate formation of HMP-P (Watanabe *et al.*, 1977). The *thiD* gene is

reported to encode HMP-P kinase which catalyses the reaction from HMP-P to HMP-PP (Imamura & Nakayama, 1981). To determine the effect of the *pdxL* and the *thiJ* mutations on the stepwise phosphorylation of HMP in intact cells, the relative rates of formation of HMP-P and HMP-PP from HMP were measured in a series of different strains including a *pdxL*⁺ *thiJ* transductant (Table 3). The results showed that in reaction mixtures containing cell suspensions of either strain SN372L-3 or strain SN372L-3-9, both HMP-P and HMP-PP were formed from HMP which had been taken up into the cell, whereas in strain SN372L-3-9-10 neither of the two phosphate esters were found, though the exogenously added HMP was detected within the cell. However, the inability of the latter strain to produce HMP-PP could not be considered to be direct evidence for the loss of HMP-P kinase, since it carries mutations in *pdxL* and *thiJ*. Subsequently, two types of transductants that had acquired the wild-type allele corresponding to the *pdxL* or *thiJ* mutation were constructed with strain SN372L-3-9-10 as recipient. Experiments with cell suspensions of two different types of transductants, M3-9-6 (*pdxL thiJ*⁺) and K3-9-101 (*pdxL*⁺ *thiJ*), revealed that the ability to carry out the stepwise phosphorylation of HMP was restored by introducing the wild-type allele corresponding to the *thiJ* mutation. Introducing the wild-type allele (*pdxL*⁺) resulted in the restoration of the ability to phosphorylate HMP into HMP-P, but not to form HMP-PP from HMP-P (Table 3). Subsequently, we noted the *thiC* (Ts) mutation carried by strain SN372L-3-9-10 and found that the strain could grow on minimal medium containing PAL at 25 °C at a rate comparable to that of strain SN372L. This indicates that the *thiC*⁺ *thiJ* mutant can synthesize thiamin-PP through the *de novo* synthetic pathway. It thus appears that free HMP is not involved in the *de novo* synthesis of thiamin-PP and that HMP-P might be the first pyrimidine compound synthesized endogenously. These results are consistent with those of Yamada & Kumaoka (1982), who showed that in *E. coli* B the C-5 and hydroxymethyl carbons of HMP are directly derived from a two-carbon fragment consisting of 4', 5' carbon atoms of the phosphoribosyl moiety of 5-aminoimidazole ribotide. The failure to isolate *pdxL* mutants from strains prototrophic for POL also suggests that the phosphorylation of POL might not be involved in the *de novo* synthesis of PAL-P, and hence POL-P is probably the first pyridine compound arising in the pathway.

Genes involved in thiamin-PP biosynthesis have been shown to be located on the chromosome in four distinct regions. Mutations in five of the genes (*thiCEFGH*), all of them involved in the biosynthesis of the pyrimidine and thiazole moieties of thiamin, have been located at 90 min on the *E. coli* genetic map. Since these genes are tightly linked to each other and coordinately expressed, they are expected to constitute the *thi* operon (Horn *et al.*, 1993). Therefore, the interaction of the *thi* operon with the *thiD*, *M* and *J* cluster, which are distant from each other on the chromosome, must be defined in order to understand the genetic control of thiamin-PP biosynthesis in *E. coli* K-12.

ACKNOWLEDGEMENTS

We thank Dr D. D. S. Smith, Institute of Food Research, Reading, UK, for his help with preparation of the manuscript.

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Received 26 March 1996; revised 25 June 1996; accepted 2 July 1996.