color development and inhibited it at least quantitatively at high concentrations. Therefore, samples containing high ATP concentrations (> 1.2 mmole/l) must be diluted prior to the phosphate determination. A similar inhibitory effect on the formation of molybdenum blue has already been reported for citric acid[5,9], tartaric acid[9], mannitol[5,12] and for other compounds[5,12]. Analogous to ATP, these substances inhibited the reaction depending on their concentration.

The impairment of maximal color production by high concentrations of ATP suggests that the inhibition of Na-K-ATPase by excess substrate as reported previously[13] might be due to an impaired color development by excess ATP and not to an inhibition of the enzyme. This assumption is supported by the similar course of enzymatic activity[13] and of optical density as observed in the present study as a function of the ATP concentration. On the other hand, a spontaneous hydrolysis of ATP could be demonstrated and previously it was reported that the cleavage of certain labile phosphates is catalyzed by molybdenum[5,10,14]. In the present study the hydrolysis of ATP was a first order reaction and the amount of inorganic phosphate formed was directly related to the concentration of ATP and to the incubation period during color development. Therefore, in samples containing readily hydrolysable phosphate esters, falsely high concentrations of inorganic phosphate might be obtained. Thus, in the presence of hydrolysable organic phosphate esters the incubation period for color development should be as short as possible. Additionally, by measuring the optical density at intervals and extrapolating back to zero-time or by subtracting blank values from parallel ATP-containing incubation mixtures (without enzyme) the true amount of inorganic phosphate present or enzymatically liberated can be determined. This is possible since spontaneous hydrolysis is a linear function of the incubation period provided it is not performed for a prolonged time resulting in a measurable decrease of the concentration of organic phosphate esters.

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thiD locus of *Escherichia coli*

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Summary. A mutant strain of *Escherichia coli* K-12 lacking phosphomethylpyrimidine kinase activity was produced from the polyauxotrophic female strain, JC1552. The locus of its lesion, for which we propose the designation *thiD*, was mapped at about 46 min on the chromosome.

Four *thi* mutations, *A*, *B*, *C*, and *O*, each affecting a different step in thiamine biosynthesis and its control mechanism, have been identified and mapped within the small region of the chromosome of *Escherichia coli* K-12[1-3]. This leads to the assumption that the genes operating in the biosynthesis of TPP (thiamine pyrophosphate), a coenzyme form of thiamine, might constitute the *thi* operon. Our previous experiments have revealed that in *E. coli* TPP is formed by the direct phosphorylation of thiamine monophosphate, the first thiamine compound synthesized from hydroxymethylpyrimidine pyrophosphate and hydroxyethylthiazole monophosphate[4,5]. A conversion from hydroxymethylpyrimidine monophosphate to the pyrophosphate, catalyzed by phosphomethylpyrimidine kinase, has been thought to be involved as an essential step in the de novo synthetic pathway of TPP. This paper describes a new locus designated as *thiD*, that affects phosphomethylpyrimidine kinase activity. The results showed that the locus is situated at a different chromosomal site from the 4 known *thi* genes already mapped, at about 90 min.

Materials and methods. The strains used in this work are all derivatives of *E. coli* K-12. A mutant strain, JC1552B5, lacking phosphomethylpyrimidine kinase was produced

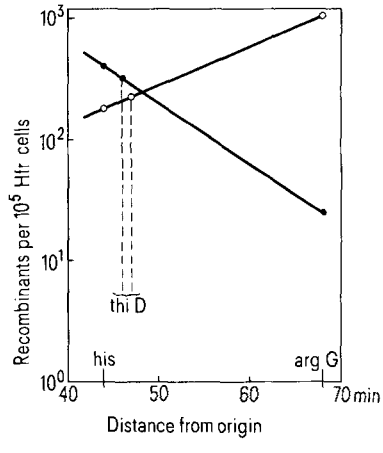

Figure 1. Location of *thiD* locus on the *Escherichia coli* chromosome by the gradient of transmission using Hfr KL209 (○——○) and AB259 (●——●) as donors. JC1552B5 was mated with the male strains for 100 min. The number of Thi^+ recombinants was plotted on the straight line drawn through the points for His^+ and Arg^+ recombinants.

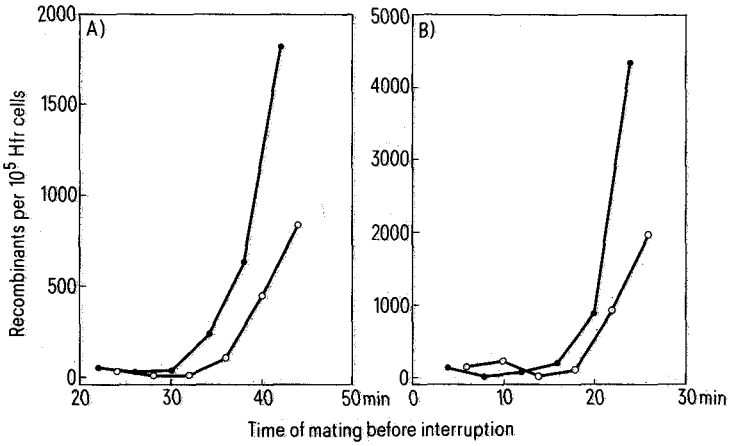

Figure 2. Results of an interrupted mating experiment using JC1552B5 as recipient with Hfr KL16 (A) and KL983 (B) as donors. The number of Thi^+ (●—●) and His^+ (○—○) recombinants were determined as a function of time between the start of mating and blending. Experimental conditions were essentially the same as those of Low[8].

Activities of enzymes involved in TPP biosynthesis from hydroxymethylpyrimidine and hydroxyethylthiazole in crude extracts of *Escherichia coli*

Enzymes	Activities* JC1552	JC1552B5
Hydroxymethylpyrimidine kinase	1.59	1.40
Phosphomethylpyrimidine kinase	0.47	0
Hydroxyethylthiazole kinase	0.45	0.44
Thiamine-phosphate pyrophosphorylase	2.02	1.62
Thiamine monophosphokinase	1.15	1.01
Thiamine monophosphate kinase	0.11	0.15

* The activities were expressed as nmoles of the product formed/mg of protein/30 min.

from the polyauxotrophic female strain, JC1552 (relevant genotype: *argG6, metB1, his-1, leu-6, trp-31, strA104,* and λ^-), by the procedure described in a preceding paper[6]. Thiamine is required by the mutant organism as the intact form because the cell membrane is not permeable to hydroxymethylpyrimidine pyrophosphate[7]. The mutation on the chromosome which affected the phosphomethylpyrimidine kinase activity was located by experiments on the gradient of transmission of the wild-type allele from Hfr to the recipient, and interrupted mating with a similar system[8]. Phage P1-mediated transduction was also tried to elucidate its position more accurately.

Results and discussion. The activities of 6 enzymes involved in TPP synthesis from hydroxymethylpyrimidine and hydroxyethylthiazole were assayed with crude extracts prepared from the parent and mutant cells. The results (table) showed that both extracts differed in their properties only with respect to phosphomethylpyrimidine kinase; the enzyme was active in the parent but inactive in the mutant. Mapping experiments on print-matings with a set of Hfr strains, which each has a special point of origin and can transfer its chromosomal markers in either clockwise or counterclockwise polarity, indicated that the mutation responsible for the enzyme deficiency was located in the chromosomal region somewhere between 43 and 51 min; these positions correspond to the points of origin of PK191 and KL983, respectively. Subsequently, JC1552B5 was mated with the 2 Hfr strains, KL209 and AB259 for 100 min without interruption. The number of recombinants produced on the selective plates containing streptomycin were scored and compared to find out the gradient of transmission of His^+, Thi^+, and Arg^+ traits from the Hfr to the mutant. The results presented in figure 1 indicated that *thiD* was located on the chromosomal position between 46.0 and 47.0 min. The location for *thiD* was also confirmed by the fact that 96.0% of $thiD^+$ recombinants isolated from the mating mixture of the mutant and AB259 had received simultaneously the unselected marker for His^+, whereas only 3.0% of them showed the Arg^+ trait. Interrupted mating experiments involving JC1552B5 and either Hfr KL16 or KL983 revealed that the wild-type allele corresponding to the *thiD* entered 2 min before *his* (fig. 2, A and B). To locate *thiD* more precisely, its cotransduction with several nearby genes was tested. In the transduction cross with AB259 as donor and JC1552B5 as recipient, no transductants for $thiD^+$ were detected among 100 his^+ transductants. Negative results were also obtained between *gyrA* and *thiD* when PCO254 *gyrA* was used as donor. Although the number of transductants tested was not large, these results could be explained if one assumed that the *thiD* was situated just in the middle between *his* and *gyrA*. Since the latter 2 genes are 3.7 min apart on the genetic map[9], one would not expect to obtain cotransduction between *thiD* and them at a high frequency. However, a cotransduction gap appears to be involved in the 40-min region and the possibility of a gap not spanned by cotransduction between *udk* and the *mgl* cluster has been discussed[9]. Efforts to obtain cotransduction of *metG* with *fpk* and *gyrA* have not been successful either[10]. Therefore, it seems that the failure to obtain cotransduction of one marker with others in this region does not immediately mean that their positions are farther away from each other than the maximal length of the chromosomal fragment carried by a single phage particle.

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