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NOTES

Genetic Mapping in *Escherichia coli* of *tmk*, the Locus for dTMP Kinase

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The genetic location of *tmk*, the gene for dTMP kinase, has been mapped at min 24.0 on the *Escherichia coli* map.

An *Escherichia coli* mutation was recently described that affects the properties of dTMP kinase (4), which is the last unique enzyme in the pathway leading to dTTP (7). The gene (*tmk*) for this essential enzyme was mapped at min 30, contradicting work we have done isolating deletions that removed this region (5, 6). We have done further mapping of *tmk* with various bacterial strains (Table 1) to resolve this, and we report here experiments which demonstrate that *tmk* is located at min 24.0.

Tests for cotransduction by bacteriophage P1 and transfer of episome F123 both indicated that *tmk* was not located between min 27 and 31. With *tmk*$^+$ donor strains that contained either *trpB*::Tn*10* (min 27.7), *zcj-2*::Tn*10* (min 29), or *zda-231*::Tn*10* (min 30), and TD105 (*tmk-1*) as the recipient, *tmk*$^+$ was never recovered in recombinants selected for Tc^r. The construction of merogenotes harboring F123, which contains the region from min 27 to 31 (2, 8), also failed to alter the Tmk$^-$ phenotype of TD105.

Since *tmk* was not in the interval between min 27 and 31, interrupted matings with Hfr KL96 were conducted to identify the region where it was located. This was done by first mapping a Tn*10* insertion isolated by Daws and Fuchs (4). This insertion was called *zcj-297*::Tn*10*, and it showed 56% cotransduction with *tmk-1*. For these crosses we used a derivative of KL96 which contained this Tn*10*, and the recipient was PLK457. The point of origin of Hfr KL96 is at min 45, and it transfers the chromosome in a counterclockwise direction (2). The data demonstrated that Tn*10* was transferred 5 min after *trp*, which corresponds to a position of approximately min 23 on the genetic map (unpublished data).

Further transductions demonstrated that Tn*10* was located near *pyrC*, which is at min 23.4 (1). P1 grown on TD405 was used to transduce MA1008 to Tc^r, and 28% (18/64) of the Tc^r transductants were also *pyrC*$^+$. In a different cross (Table 2), the cotransduction between Tn*10* and *pyrC* was 54% (112/208). Taken together (130/272, 48% cotransduction), these data indicate that the Tn*10* is 0.45 min from *pyrC* (9). To determine on which side of *pyrC* the Tn*10* was located, we used P1 grown on TD405 to transduce RR274 to Tc^r. This recipient was *ptsG*, which maps at min 24.4 (1), and BCIG (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) plates were used to test for *ptsG* (3). Forty-one percent (90/221) of the Tc^r transductants were *ptsG*$^+$, which demonstrated that Tn*10* was located 0.5 min from *ptsG*, midway between *pyrC* and *ptsG*. Based on its location (min 23.9), this Tn*10* should be designated *zcd-297*::Tn*10*.

Once the insertion site of the Tn*10* had been determined, we mapped the location of *tmk*. Daws and Fuchs (4) reported that these loci exhibited 56% cotransduction, which is similar to the value of 61% that we obtained. To determine the location of *tmk* relative to *zcd-297*::Tn*10* and *pyrC*, a three-factor cross was conducted (Table 2). The data demonstrate that the genetic sequence is *pyrC–zcd-297*::Tn*10–tmk*. The 37% cotransduction between *pyrC* and *tmk* indicates that the distance between these two loci is 0.6 min. This positions *tmk* at min 24.0, between *zdd-297*::Tn*10* and *ptsG*.

In the genetic mapping described above, resistance to 2′-3′-dideoxythymidine (20 μg/ml) was used as the criterion for the presence of *tmk*. Although very useful, this is an indirect test and is dependent on other genetic loci that decrease the thymidine nucleotide pool (4). As a more direct test, we have also determined the enzyme activity of dTMP

TABLE 1. Bacterial strains used

Strain	Genotype	Source or reference
MA1008	Hfr *thi-1 pyrC46 relA1 lacZ43*	W. Maas via CGSC[a]
PLK457	F$^-$ *trpR trpA9605 his-29 ilv pro arg thyA deoB* or *deoC tsx*	This laboratory
PLK2013	As MA1008, except *zcd-297*::Tn*10*	P1(TD405) × MA1008, Tc^r selection
PLK2041	As TD105, except *zdc-297*::Tn*10 pyrC46*	P1(PLK2013) × TD105, Tc^r selection
RR274	Hfr *ptsG fhuA22*	R. Vinopal
TD105	F$^-$ *metB leu hisA lacY malA xyl mtl rpsL tpp-75 cdd-50 dcd-1 tmk-1*	T. Daws (4)
TD405	F$^-$ *metB leu hisA lacY malA xyl mtl rpsL arg tpp-75 dcd-1 zcd-297*::Tn*10*	T. Daws (4)

[a] CGSC, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.

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TABLE 2. Transductions with bacteriophage P1

Donor	Recipient	Selected marker	Genotype	No.
PLK457[a]	PLK2041[b]	*pyrC*$^+$	*pyrC*$^+$ Tc^r *tmk*$^+$	10
			pyrC$^+$ Tc^s *tmk*$^+$	66
			pyrC$^+$ Tc^s *tmk*	46
			pyrC$^+$ Tc^r *tmk*	86

[a] Relevant markers: *pyrC*$^+$ Tc^s *tmk*$^+$.
[b] Relevant markers: *pyrC zcd-297*::Tn*10 tmk-1*.

kinase in several transductants obtained from the cross described in Table 2. Using the assay procedures described by Daws and Fuchs (4), we observed that *pyrC*$^+$ Tc^s *tmk*$^+$ transductants had approximately 10 times the specific activity of dTMP kinase that *pyrC*$^+$ Tc^s *tmk* transductants had (unpublished data).

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