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Location on the Chromosome of *Salmonella typhimurium* of Genes Governing Pyrimidine Metabolism

II. Uridine Kinase, Cytosine Deaminase and Thymidine Kinase *

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Summary. The remaining unmapped genes encoding known functions of the pyrimidine salvage pathways have been located on the chromosome of *Salmonella typhimurium*. The gene, *cod*, encoding cytosine deaminase, *udk*, encoding uridine kinase and *tdk*, encoding thymidine kinase were located at 108, 69, and 53 min respectively. The following gene orders were established: *serA-metC-argE-cod-strA*; *cdd-metG-udk-his*; and *pyrF-cysB-trp-tdk-his*. The gene *tdk* lies in that region of the *S. typhimurium* chromosome which is inverted with respect to *E. coli*; its mapping further defines the limits of the inverted segment.

The *de novo* biosynthesis of UMP, which in turn serves as a total source of pyrimidine, is catalyzed by six enzymes encoded by unlinked genes (O'Donovan and Neuhard, 1970). But in addition auxillary or salvage pathways exist which allow the cell to incorporate exogenously supplied pyrimidines and pyrimidine nucleosides (Fig. 1). These pathways have recently been elucidated (Beck *et al.*, 1972). Genes encoding enzymes of *de novo* biosynthesis and of the salvage pathways have been mapped (Yan and Demerec, 1965; Beck and Ingraham, 1971) with the exception of those encoding uridine kinase (*udk*), cytosine deaminase (*cod*) and thymidine kinase (*tdk*). In this paper we establish the location on the *S. typhimurium* chromosome of these three genes.

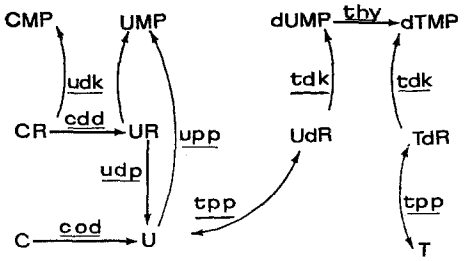

Fig. 1. Salvage pathways for pyrimidine bases and nucleosides. Gene designations are: *cdd* cytidine deaminase, *cod* cytosine deaminase, *tdk* thymidine kinase, *tpp* thymidine phosphorylase, *udk* uridine kinase, *udp* uridine phosphorylase

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Materials and Methods

Media and genetic manipulation are as previously described (Beck and Ingraham, 1971). P1 mediated transduction was done by the method of B. A. D. Stocker (personal communication) utilizing rough strains of *S. typhimurium*. Strains used and their genotype are listed in Table 1.

Table 1. Strains used

Strain number	Genotype
JL409	*ara⁻, cod-101, pyrC7*
JL415	*cod-8, cdd-9, pyrC1502, udk-6, udp-8, upp-101*
JL417	Hfr K3, *serA13, glpD*
JL426	Hfr K3, *his⁻, metC30, strA*
JL428	*cod-101, glpD, pyrC7, serA13*
JL435	*cod-8, cdd-9, gal⁻, pyrA81, pyrC1502, udk-6, udp-8, upp-101, ORO⁺*[a]
JL436	*pyrC7, cod-101, metC30, strA*
JL440	Hfr K3, *serA13, argE, trp⁻*
JL625	Hfr B2, *arg⁻, pro⁻*
JL629	Hfr K5, *serA13*
JL885	*pyrC7, cod-101, argE, strA*
JL891	*hisE35, metG319, gal⁻, strA*
JL1018	*pyrF146*
JL1221	*argB69, cod-8, cdd-9, pyrC1502, tdk-1, tpp-1, udp-11*
KP1236	*guaB, pyrE125, trp⁻*
JL1238	*cod-8, cdd-9, pyrC1502, tdk-1, thy-1392, tpp-1, udp-11*
JL1250	*cod-8, cdd-9, pyrC1502, tdk-1, thy-1392, tpp-1, trp⁻, udp-11*

[a] Strain is able to utilize orotic acid (10 μg/ml) as pyrimidine source.

Results and Discussion

Mapping of the Gene for Uridine Kinase: udk. There are two pathways by which *S. typhimurium* converts uridine to UMP (a) UR $\xrightarrow{udk}$ UMP[1] (b) UR $\xrightarrow{udp}$ U $\xrightarrow{upp}$ UMP (Beck *et al.*, 1972). Thus, selection and phenotypic recognition of mutations in the *udk* gene cannot be done in simple pyrimidine auxotrophs; rather a more complicated genetic background is required. We employed a heat-sensitive lesion in *pyrC* to preclude *de novo* biosynthesis combined with mutational blocks in *upp* and *udp*. In such a strain (*pyrC, udp, upp*) selection for resistance to the analogue, FUR, yields mutants in *udk*. Such mutants are unable to grow at 42° C on any medium, owing to inability to synthesize UMP by the *de novo* pathway and to utilize exogenous U or UR. At 30° C however they grow well on unsupplemented minimal medium. The mutation in *udk* carried by JL415 has been shown to result in loss of uridine kinase activity (Beck *et al.*, 1972). However, it proved difficult to use strain JL415 in genetic experiments since with this strain all selections for *udk⁺* recombinants had to be done at 42° C, a temperature at which scoring was difficult. In order to construct a strain in which scoring of *udk⁺*

1 Abbreviations: *C* = cytosine; *CMP* = Cytidine monophosphate; *CR* = cytidine; *dUMP* = 2′-deoxyuridine monophosphate; *dTMP* = 2′-deoxythymidine monophosphate; *FC* = 5-fluorocytosine; *FUdR* = 5-fluoro-2′-deoxuridine; *FU* = 5-fluorouracil; *FUR* = 5-fluorouridine; *T* = thymine; *U* = uracil; *UdR* = 2′-deoxyuridine; *UMP* = uridine monophosphate; *UR* = uridine; *UTP* = uridine triphosphate.

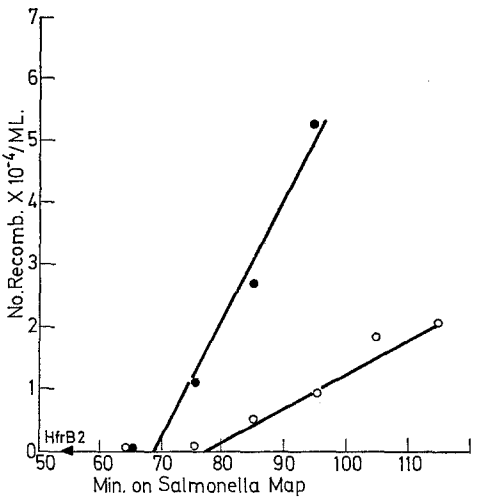

Fig. 2. Interrupted mating to determine the location of the *udk* gene. Hfr B2 (JL625) was mated with JL435 and *udk*$^+$ (●) and *upp*$^+$ (○) recombinants were selected. Recombinants were scored as follows: samples of the mating mixture were distributed on (A) plates containing uridine and (B) plates containing uracil. Recombinants growing on uracil plates are *upp*$^+$. Those growing on uridine plates are *upp*$^+$ or *udk*$^+$. The number of *udk*$^+$ recombinants was determined by difference. The ordinate is the number of recombinants per ml and the abscissa a segment of the *S. typhimurium* map (Sanderson, 1970)

recombinants could be done at 37° C, we selected a spontaneous mutation which allowed JL415 to utilize exogenous orotate as its pyrimidine source (ORO$^+$). Subsequently this strain was made pyrimidine requiring at all temperatures by introducing the deletion *pyrA81*[2]. The resulting strain, JL435, has an absolute requirement for orotate at all temperatures, which cannot be replaced by uracil or uridine.

This strain was used for interrupted mating experiments with various Hfr's. A functional *udk* gene was transferred early in matings with Hfr B2 (Fig. 2) placing *udk* at about 13 min from the origin of this Hfr. With Hfr K5, however, *udk* was not an early marker, indicating that *udk* lies counterclockwise from the origin of Hfr K5 (Fig. 3). This general localization of *udk* was confirmed by establishing cotransduction between *udk* and the *his* operon as well as with *metG* using phage P1 (Table 2). These data place *udk* between the histidine operon and *metG*; *metG* in turn cotransduced with another gene governing the pyrimidine salvage pathway, *cdd*, as shown in Fig. 3. No cotransduction could be detected between any of these markers using phage, P22.

In addition, we found that the *Escherichia coli* episome F′ 32 does not complement *udk*, but is known to cover *metG* (Fink and Roth, 1968).

Mapping of the Gene for Cytosine Deaminase: cod. Cytosine deaminase catalyzes the hydrolytic deamination of cytosine to form uracil (Fig. 1). Strains lacking cytosine deaminase are unable to grow on cytosine but can grow on uracil as a

2 The introduction of *pyrA81* was done in two steps. An *ara*$^-$ mutation was selected in JL415 ORO$^+$ and subsequently transduced to *ara*$^+$, *pyrA81*.

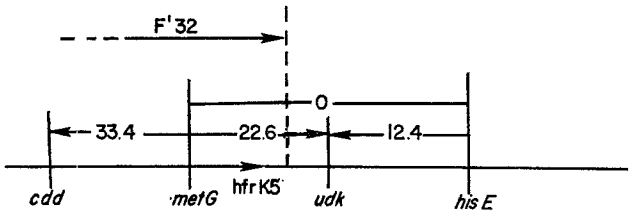

Fig. 3. Detailed genetic map of *S. typhimurium* in the *his-metG* region. The numbers between markers refer to cotransduction frequencies determined with phage P1 (Table 2). Arrows point in the direction of the selected donor marker. The cotransduction frequency between *metG* and *udk* was determined in an F$^-$ strain

Table 2. Mapping of *udk* by transduction with phage P1

Recipient	Transductional donor	Number *udk*$^+$ transductants[a]	Unselected markers	% cotransduction
JL435	JL891	281	*metG*$^-$	22.6
		274	*hisE*$^-$	12.4
		254	*metG*$^-$*hisE*$^-$	0

[a] The *udk*$^+$ transductants were scored as described in the legend of Fig. 2.

pyrimidine source (Beck *et al.*, 1972). Mutants defective in cytosine deaminase may be selected in two ways: a) in pyrimidine prototrophs by resistance to the cytosine analogue, FC; amongst this group those sensitive to FU are *cod*$^-$; b) in pyrimidine auxtorophs by inability to utilize cytosine as a sole pyrimidine source (by mutagenesis and subsequent penicillin counterselection). Strain JL409, used in most genetic experiments was isolated by the latter method and shown by enzyme assay to contain less than 1% of the wild type activity (Beck, unpublished data).

The approximate location of *cod* was determined by interrupted matings of various Hfrs with JL409. Hfrs K3 and K10 both donate *cod* as an early marker. Interrupted matings between Hfr K3 and JL409 (Fig. 4) placed the *cod* gene at about 108 minutes on the *S. typhimurium* map. A second cross (not shown) between Hfr K2 and JL409 gave similar results.

In order to locate *cod* more precisely, Hfr mediated crosses were made using multiply-marked parents (Table 3). Cross 1 established the probable gene order *argE-cod-strA* since among the *argE*$^+$, *strA*$^+$ recombinants *cod*$^+$ was a much more frequent class than *cod*$^-$. In cross 2 the greater frequency of *argE*$^-$ than *argE*$^+$ recombinants indicates that *metC* lies to the left of *argE*. This conclusion is substantiated by cross 3 where we found the *argE*$^-$ class less frequent than *argE*$^+$ among the *cod*$^+$, *metC*$^-$ recombinants. Finally, in cross 4, the order *serA*, *metC*, *cod* is indicated by the greater frequency of *metC*$^-$ recombinants. Thus we conclude that the gene order in this region of the chromosome is: *serA-metC-argE-cod-strA*. Using phage P22, attempts were made to detect cotransduction between *cod* and each of the following markers *serA*, *metC*, *argE*, *strA*, *aroB* and *aroC* (see Sanderson, 1970); all gave negative results.

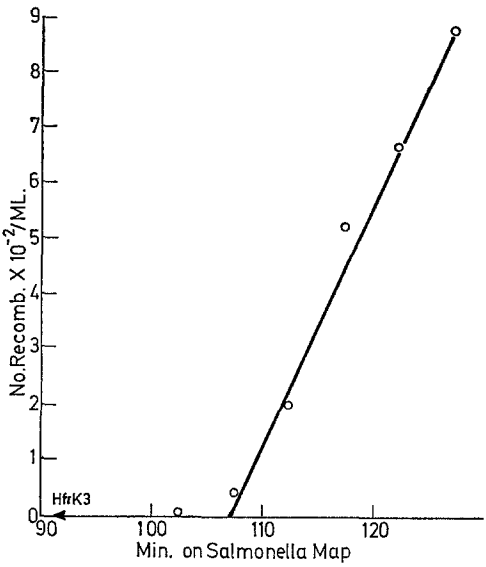

Fig. 4. Mapping of *cod* by interrupted mating. The abscissa represents a fragment of the *S. typhimurium* map. The ordinate is the number of *cod*$^+$ recombinants

Phenotype of the tdk Mutation. We have previously described the isolation of *S. typhimurium* mutants lacking thymidine kinase (Beck *et al.*, 1972). The selection was based on the observation that FUdR is toxic to *S. typhimurium* for two reasons: 1. it is phosphorylated by thymidine kinase to 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP), which is a potent inhibitor of thymidylate synthetase (Cohen *et al.*, 1958); and 2. it is catabolized by the inducible enzymes thymidine phosphorylase (*tpp*) and uridine phosphorylase (*udp*) to FU (Fig. 1). The latter route for toxicity may be counteracted phenotypically by adding uracil, which competes effectively with the FU formed. Thus, *tdk* mutants may be obtained by selecting for FUdR-resistance in a *tpp*$^-$, *udp*$^-$ strain or in the presence of uracil a *tpp*$^-$, *udp*$^+$ strain may be used. In a *tpp*$^+$ background FUdR is broken down too rapidly to maintain toxic intracellular concentrations.

Lack of thymidine kinase does not result in a scorable phenotype other than resistance to FUdR, a character which is not very useful for genetic analysis. However, thymidine kinase is essential for bacterial strains that carry a mutation in thymidylate synthetase (*thy*); such strains are entirely dependent on exogenous thymine or thymidine for growth (Fig. 1). Accordingly, a mutation that confers heat-lability on the enzyme thymidylate synthetase (*thy-1392*) was transduced into the *tdk* mutant JL1221, taking advantage of the cotransducibility of *argB* and *thy* (Eisenstark *et al.*, 1968). The resulting strain, JL1238 (Table 1), which has the genotype *tdk-1*, *thy-1392* is prototrophic at 30° C and is unable to grow at 42° C even in the presence of thymidine.

Mapping of tdk. For genetic studies we used the lack of growth of JL1238 at 42° C. Recombinants selected for their ability to grow on thymidine-containing plates at 42° C were of two classes: *tdk*$^+$, *thy*$^-$ or *tdk*$^-$, *thy*$^+$, which can easily be

Table 3. Mapping of *cod* relative to neighbouring markers by crosses with Hfrs

Cross	Recipient	Donor Hfr	Selected markers	No. of recomb.	Unselected markers	% coinheritance
1	JL885	JL417	*argE*$^{+}$[a]	350	*cod*$^{+}$*strA*$^{+}$	42
					cod^{-}*strA*$^{+}$	1.1
					cod^{+}*strA*$^{-}$	37.4
					cod^{-}*strA*$^{-}$	19.5
2	JL436	JL440	*metC*$^{+}$*cod*$^{+}$*strA*$^{-}$[b]	186	*argE*$^{-}$	88
					argE^{+}	12
3	JL885	JL426	*cod*$^{+}$[c]	200	*metC*$^{+}$*argE*$^{+}$	62
					metC^{+}*argE*$^{-}$	11.5
					metC^{-}*argE*$^{+}$	24.5
					metC^{-}*argE*$^{-}$	2
4	JL428	JL426	*serA*$^{+}$*cod*$^{+}$[d]	62	*metC*$^{-}$*strA*$^{-}$	42
					metC^{-}*strA*$^{+}$	44.6
					metC^{+}*strA*$^{-}$	4.8
					metC^{+}*strA*$^{+}$	8

[a] Indicated gene order:

Hfr origin				
	argE^{+}	*cod*$^{+}$	*strA*$^{+}$	donor
				recipient
	argE^{-}	*cod*$^{-}$	*strA*$^{-}$	

[b] Indicated gene order:

Hfr origin					
	metC^{+}	*argE*$^{-}$	*cod*$^{+}$	*strA*$^{+}$	donor
					recipient
	metC^{-}	*argE*$^{+}$	*cod*$^{-}$	*strA*$^{-}$	

[c] Indicated gene order:

Hfr origin				
	metC^{-}	*argE*$^{+}$	*cod*$^{+}$	donor
				recipient
	metC^{+}	*argE*$^{-}$	*cod*$^{-}$	

[d] Indicated gene order:

Hfr origin					
	serA^{+}	*metC*$^{-}$	*cod*$^{+}$	*strA*$^{-}$	donor
					recipient
	serA^{-}	*metC*$^{+}$	*cod*$^{-}$	*strA*$^{+}$	

distinguished on the basis of their ability to grow in the absence of thymidine at 42° C.

The structural gene for thymidine kinase has been previously mapped in *E. coli*. It was found to be located close to the tryptophan operon. Using phage P1, *tdk* was shown to cotransduce with *gal U*, *trp* and *cysB* with decreasing frequencies (Igarashi *et al.*, 1967). The gene order determined for *E. coli* is shown

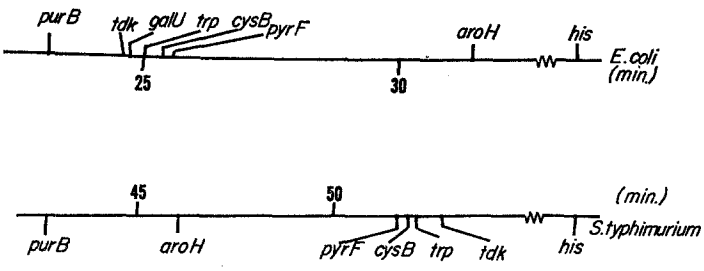

Fig. 5. Comparison of the segments of the *E. coli* and *S. typhimurium* chromosomes in the *tdk* region. Numbers refer to minutes on the standard maps (Taylor, 1970; Sanderson, 1970). Gene designations are: *aro* aromatic amino acids, *cys* cystein, *gal* galactose, *pur* purine, *pyr* pyrimidine, *tdk* thymidine kinase, and *trp* tryptophan

Table 4. Transductional mapping of *tdk*

Recipient	Donor	Selection	No. of transductants	Genotypes of transductants		
				thy$^+$, *tdk*$^-$	*tdk*$^+$, *thy*$^-$	
					trp$^+$	*trp*$^-$
JL1238	KP-1236	growth at 42° C on min + TdR + tryptophan	264	118	141	5

in Fig. 5. The segment of the chromosome containing the genes *trp*, *cysB* and *pyrF* has been found to be inverted on the *S. typhimurium* chromosome as compared with that of *E. coli* (Sanderson and Hall, 1968) but the extent of the inversion is unknown. Thus it was of interest to determine whether the gene for thymidine kinase in *S. typhimurium* maps in this region, and if so, whether it falls on the inverted segment.

In preliminary experiments, JL1238, which is unable to grow on any medium at 42° C (see above), was transduced to grow on minimal plates containing tryptophan and thymidine at 42° C, using phage P22 grown on KP1236 (*trp*$^-$). As shown in Table 4, two classes of transductants were obtained; those that received a *thy*$^+$ gene and those that received a *tdk*$^+$ gene. In addition the data show cotransduction between *trp* and *tdk* indicating that *tdk* in *S. typhimurium* is located in the same general region as in *E. coli*. To determine the location of *tdk* with respect to *trp*, a *trp*$^-$ derivative of JL1238 was constructed: JL1250. Table 5 shows the results of transduction of JL1250 to *trp*$^+$ (at 30° C) using phage P22 grown on a *pyrF*$^-$ strain (JL1018). The transductants were scored for coinheritance of the *tdk* and *pyrF* genes. Table 5 confirms the linkage of *tdk* and *trp* (approximately 4% cotransduction). Since all *trp*$^+$, *pyrF*$^-$ transductants from this cross are *tdk*$^-$, *tdk* is not between *trp* and *pyrF*; further it is not located to the left of *pyrF* since all *trp*$^+$, *tdk*$^+$ transductants are *pyrF*$^+$. Thus, the gene order on the *S. typhimurium* chromosome is: *pyrF-trp-tdk*. By comparing this order with that of *E. coli* (Fig. 5) it is apparent that *tdk* lies on the inverted segment, which extends at least from *aroH* to *tdk*.

Table 5. Localization of *tdk*[a]

Recipient	Donor	Selected marker	No. of transductants	Unselected markers			
				pyrF$^+$*tdk*$^-$	*pyrF*$^+$*tdk*$^+$	*pyrF*$^-$*tdk*$^-$	*pyrF*$^-$ *tdk*$^+$
JL1250	JL1018	*trp*$^+$ at 30° C	264	211	12	41	0

[a] Indicated gene order:

pyrF$^-$ *trp*$^+$ *tdk*$^+$ donor

recipient

pyrF$^+$ *trp*$^-$ *tdk*$^-$

Since the origin of Hfr B2 is in the region of *tdk* (Sanderson, 1970), it was of practical interest to determine whether *tdk* is an early marker for Hfr B2. By crossing Hfr B2 with strain JL1238 we found that *tdk* was not an early marker.

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