# A DEOXYTHYMIDINE KINASE DEFICIENT MUTANT OF *ESCHERICHIA COLI*. II. MAPPING AND TRANSDUCTION STUDIES WITH PHAGE $\phi 80$[1]

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A mutant of *Escherichia coli* unable to incorporate external $C^{14}$-deoxythymidine (TdR) into its DNA has been isolated recently and was found to lack the activity of deoxythymidine kinase that catalyzes the conversion of TdR to deoxythymidine 5′-monophosphate (dTMP) (HIRAGA, IGARASHI and YURA 1967). The mutant grows normally in the medium without TdR, since the organism can synthesize dTMP and thus deoxythymidine 5′-triphosphate (dTTP) endogenously. When the endogenous synthesis of dTMP is inhibited by fluorouracil (FU) or fluorodeoxyuridine (FUdR), the growth of wild-type *E. coli* becomes dependent on externally supplied TdR as well as uridine. Since the mutant lacking TdR kinase activity cannot utilize external TdR, such a mutant would be expected to be incapable of growth in the medium containing FU or FUdR even in the presence of TdR and uridine. In agreement with this expectation, the mutant failed to grow on this medium, in contrast to the wild-type strain. The biochemical basis of this finding has been discussed elsewhere (HIGARA, IGARASHI and YURA 1967). At any event, this character is important in the genetic analysis of the mutant presented below, because it provides a convenient means not only of identifying the *tdk* mutant allele from the wild-type $tdk^+$ allele among recombinant clones but also of selecting $tdk^+$ clones in a population of *tdk* mutant bacteria.

In this paper, it will be shown that the mutant characteristics described above are caused by mutation of a single gene (designated as *tdk*) located near the cluster of the tryptophan structural genes on the *E. coli* chromosome. The $tdk^+$ gene has also been found to be transduced by phage $\phi 80$, and some of the characteristics of the transduction system will be reported. An abstract of a part of this work has appeared elsewhere (IGARASHI, HIRAGA and YURA 1966).

## MATERIALS AND METHODS

*Bacterial and phage strains:* The bacterial strains (*E. coli* K-12) used and their genetic characters are listed in Table 1. The *tdk-1* mutant (strain KY608) was originally isolated as a mutant that differs from the parental strain W3110 with respect to the four characters; *tdk*, *ts*, *tonB* and *trpE* (HIRAGA. IGARASHI and YURA 1967). In all experiments reported here, however, a $ts^+$ revertant derived from this strain with other appropriate markers were used. Phage $\phi 80$, $\phi 80dt_0$ and $\phi 80pt_1$ (MATSUSHIRO 1963; MATSUSHIRO, SATO and KIDA 1964) were obtained from DR. A.

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TABLE 1

*Bacterial strains used and their genetic characters**

| Strain | Genetic characters | Origin |
|---|---|---|
| W3110 | F⁻ : K-12 wild type | |
| KY608 | F⁻ : *tdk-1 ts trpE tonB* | W3110 |
| KY688 | F⁻ : *tdk-1 ilv trpE tonB* | KY608 |
| KY830 | Hfr : *thr leu his met gal lac mal xyl mtl ara thi tonA lam str azi* | PA678 |
| KY893 | Hfr : *tdk-1 ilv tonB* | KY688 |
| KY894 | F′ : *tdk-1 ilv trpE tonB*/F′trp | 5TD4 × KY688 |
| KY895 | F⁻ : *tdk-1 ilv* | KY484—× KY688 |
| KY896 | F⁻ : *tdk-1 ilv* (*ϕ*80) | KY895 |
| KY904 | F⁻ : *tdk-1 ilv* (*ϕ*80) (*ϕ*80d*tdk*+) | KY895 |
| W4597 | *UDPG* | FUKASAWA |
| KY976 | *UDPG* (*ϕ*80) | W4597 |
| KY980 | F⁻ : *tdk-1 ilv trpE tonB* (*ϕ*80) | KY899 |
| KY981 | F⁻ : *tdk-1 ilv* (*ϕ*80) *lam* | KY896 |
| KY982 | F⁻ : *tdk-1 ilv lam* | KY895 |
| KY123 | F⁻ : *gal* (λ) *lam* | W3102 |
| KY124 | F⁻ : *gal tonB* (*ϕ*80) | W3102 |
| KY484 | F⁻ : *cysB* | Y-mel |
| KY496 | F⁻ : *his cysB trpC str* | 5TD4 (FREDERICQ) |

* Gene symbols: Genes determining biosynthesis; *cys*, cysteine; *his*, histidine; *trp*, tryptophan; *ilv*, isoleucine and valine; *thr*, threonine; *leu*, leucine; *met*, methionine; *thi*, thiamine. Gene determining sugar utilization; *gal*, galactose; *lac*, lactose; *mal*, maltose; *xyl*, xylose; *mtl*, mannitol; *ara*, arabinose. *UDPG* (genes determining uridine diphosphoglucose pyrophosphorylase synthesis); *str* (genes determining response to streptomycin); *azi* (genes determining response to azide); *tdk* (genes determining deoxythymidine kinase synthesis). Genes determining response to phage; *tonB*, phage T1; *lam*, phage λ; *tonA*, phage T1 and T5; (λ), (*ϕ*80), lysogenicity for phage λ or *ϕ*80, respectively; *ts* (genes determining temperature sensitive growth on a peptone-glucose medium. A sexual cross is represented as donor × recipient; transduction by phage P1*kc* as donor —× recipient.

MATSUSHIRO. Other phages used were P1*kc* (LENNOX 1955), λ and $\lambda b_2$ (KELLENBERGER, ZICHICHI and WEIGLE 1960).

*Media:* The following media were used. (A) Glucose-minimal medium (VOGEL and BONNER 1956). (B) Peptone-glucose medium (HIRAGA, IGARASHI and YURA 1967). (C) EMB-galactose medium (LEDERBERG 1950). Amino-acid supplements were made to a final concentration of 20 μg/ml and streptomycin was added to 100 μg/ml. To identify the *tdk* genotype as well as to select for *tdk*+ clones in a population of *tdk* mutant bacteria, a peptone-glucose medium containing FU or FUdR (25 μg/ml), uridine (25 μg/ml) and TdR (50 μg/ml) was employed (FU medium) unless otherwise indicated. The *tdk* mutant cannot grow on this medium, whereas *tdk*+ bacteria are capable of growth after 2 days incubation at 37°C.

*Matings and analyses of unselected markers:* Overnight cultures of Hfr and F⁻ strains in peptone-glucose medium were diluted 50-fold in a fresh medium and were grown to approximately $5 \times 10^8$ cells/ml. A 0.5 ml portion of a donor culture was mixed with 4.5 ml of a recipient culture in a 50 ml flask and the mixture incubated at 37°C for 150 min without shaking. An aliquot was diluted with saline, spread on appropriate selective agar plates, and incubated at 37°C for 2 days. Recombinant colonies were picked and streaked with sterile toothpicks on the same selective medium. After overnight incubation, the plates were replicated onto several appropriate media to determine the auxotrophic markers of each recombinant. In addition, cell suspensions from each recombinant were streaked on FU medium to identify the *tdk* genotype or cross-streaked against T1 phage on peptone-glucose medium to determine the *tonB* genotype.

*Transduction with phage P1*kc: An exponential-phase culture of recipient bacteria ($5 \times 10^8$ cells/ml) in peptone-glucose medium was centrifuged and resuspended in saline containing 5 mM

$CaCl_2$. Phage P1*kc* that had been multiplied on donor bacteria was added at a multiplicity of 10. After standing 20 min at 37°C, aliquots were spread onto appropriate selective agar plates that were then incubated at 37°C for 2 days. Transductant colonies were picked, purified and analyzed for unselected markers as described above.

*$C^{14}$-TdR incorporation:* To cultures of mutant *tdk* infected with an HFT lysate containing $\phi$80d*tdk*+ particles were added TdR-2-$C^{14}$ (8 $\mu$g/ml, 300 $\mu$c/mmole) and deoxyadenosine (250 $\mu$g/ml) to minimize the degradation of TdR (BOYCE and SETLOW 1962); these were incubated at 37°C with shaking. At various times, 1 ml aliquots were removed to determine the incorporation of $C^{14}$-TdR into acid-insoluble fraction as described by HIRAGA, IGARASHI and YURA (1967). In the determination of the ability of recombinants to incorporate $C^{14}$-TdR, cells were grown overnight at 37°C in 1 ml of glucose-minimal medium supplemented with appropriate amino acids (20 $\mu$g/ml), $C^{14}$-TdR (8 $\mu$g/ml, 300 $\mu$c/mmole) and deoxyadenosine (250 $\mu$g/ml), and the radioactivity in the acid-insoluble fraction was measured.

*Assay for TdR kinase:* Cell extracts were prepared by a sonic oscillator and the assay for TdR kinase was performed as described previously (HIRAGA, IGARASHI and YURA 1967).

## RESULTS

*Mapping of the* tdk *gene:* In an attempt to identify and map the *tdk-1* mutation responsible for the loss of TdR kinase activity, a bacterial cross was carried out between strains KY830 (Hfr, *tdk*+) and KY688 (F⁻, *tdk-1*). The results are shown in Table 2. Ninety-eight recombinants selected for either *his*+ *ilv*+ or

TABLE 2

*Recombinants from a cross KY830(Hfr) × KY688(F⁻)**

| Selective marker | Unselected marker | | | | Numbers of recombinants |
|---|---|---|---|---|---|
| | *tdk*† | *gal* | *trp* | *his* | |
| *his*+ *ilv*+ | 1‡ | 1 | 1 | . | 4 |
| | 1 | 0 | 1 | . | 10 |
| | 0 | 1 | 1 | . | 2 |
| | 0 | 1 | 0 | . | 6 |
| | 0 | 0 | 1 | . | 3 |
| | 0 | 0 | 0 | . | 25 |
| | | | | Total | 50 |
| *thr*+ *leu*+ *ilv*+ | 1 | 1 | 1 | 0 | 3 |
| | 1 | 0 | 1 | 1 | 2 |
| | 1 | 0 | 1 | 0 | 7 |
| | 1 | 0 | 0 | 1 | 1 |
| | 1 | 0 | 0 | 0 | 1 |
| | 0 | 1 | 1 | 0 | 1 |
| | 0 | 1 | 0 | 1 | 2 |
| | 0 | 0 | 1 | 1 | 2 |
| | 0 | 1 | 0 | 0 | 5 |
| | 0 | 0 | 1 | 0 | 3 |
| | 0 | 0 | 0 | 0 | 21 |
| | | | | Total | 48 |

* Hfr: *tdk*+ *thr leu gal trpE*+ *his ilv*+
F⁻: *tdk-1 thr*+ *leu*+ *gal*+ *trpE his*+ *ilv*
† The *tdk* marker was determined by incorporation of $C^{14}$-TdR *in vivo*.
‡ 1 represents markers from the Hfr parent and 0 represents markers from the F⁻ parent.

*thr*$^+$ *leu*$^+$ *ilv*$^+$ were tested for the ability to incorporate $C^{14}$-TdR as well as for distribution of other unselected markers. Among the *his*$^+$ *ilv*$^+$ recombinants tested, the integration of the *tdk*$^+$ marker was always accompanied by that of the *trpE*$^+$ marker (14/14). With *thr*$^+$ *leu*$^+$ *ilv*$^+$ recombinants, a close linkage between *tdk* and *trp* markers was again noted, compared to the relation with other markers tested. These results suggested that the ability to incorporate external TdR is determined by a single gene, *tdk*, which appears to be located near *trpE*.

To determine the relative position of the markers *cysB*, *trp*, *tonB* and *tdk* on the chromosome, a four-factor cross was carried out between strains KY893 (Hfr, *tdk-1*) and KY496 (F$^-$, *tdk*$^+$). In this experiment, the *tdk* genotype of the recombinants was determined first by streaking cell suspensions on FU medium, and 50 of them were then tested for the ability to incorporate $C^{14}$-TdR. All FU-resistant clones incorporated $C^{14}$-TdR, whereas none of the FU-sensitive clones did. The complete agreement between the results obtained by the two methods indicate that FU-sensitivity adequately differentiates the Tdk$^-$ and Tdk$^+$ phenotypes. It is used in all subsequent experiments. The results of this cross, summarized in Table 3, suggest that the markers are located in the order *tdk*, *tonB*, *trpC*, *cysB*.

Further support of this result was obtained by transduction experiments with phage P1*kc*, as shown in Table 4. No difficulty was encountered in scoring the *tonB* marker in this experiment despite the fact that the growth of T1 phage is restricted in P1 lysogen (LEDERBERG 1957). This may be due to the overgrowth of the modified T1 phage that had been produced in transductants that are presumably lysogenic for P1*kc*. From these results, cotransduction frequencies for the *tdk-tonB*, *tdk-trpE* and *tdk-cysB* marker pairs were calculated to be 0.75, 0.66 and 0.28, respectively.

TABLE 3

*Recombinants from a cross KY893(Hfr) × KY496(F$^-$)**

| Selective marker | Number of crossovers required in the region examined† | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | | | | 1 | | | | | 2 | | | | | 3 | | | | | |
| | *tdk*‡ | *ton* | *trp* | *cys* | | *tdk* | *ton* | *trp* | *cys* | | *tdk* | *ton* | *trp* | *cys* | | *tdk* | *ton* | *trp* | *cys* | |
| *trp*$^+$ *str* | 1§ | 1 | 1 | 1 | 471 | 0 | 1 | 1 | 1 | 9 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 |
| | . | . | . | . | . | 0 | 0 | 1 | 1 | 5 | 0 | 0 | 1 | 0 | 3 | . | . | . | . | . |
| | . | . | . | . | . | 1 | 1 | 1 | 0 | 11 | 1 | 0 | 1 | 1 | 0 | . | . | . | . | . |
| | | | | | | | | | | 25 | | | | | 4 | | | | | |
| *cys*$^+$ *str* | 1 | 1 | 1 | 1 | 424 | 0 | 1 | 1 | 1 | 13 | 1 | 0 | 1 | 1 | 3 | 0 | 1 | 0 | 1 | 0 |
| | . | . | . | . | . | 0 | 0 | 1 | 1 | 13 | 1 | 1 | 0 | 1 | 2 | . | . | . | . | . |
| | . | . | . | . | . | 0 | 0 | 0 | 1 | 45 | . | . | . | . | . | . | . | . | . | . |
| | | | | | | | | | | 71 | | | | | 5 | | | | | |

* Hfr: *tdk-1 tonB trpC*+ *cysB*+ *str*+
F$^-$: *tdk*+ *tonB*+ *trpC cysB str*
† The number of crossovers assuming that the markers are located in the order of *tdk*, *tonB*, *trpC* and *cysB*.
‡ The *tdk* marker was determined by the sensitivity to FU. In addition, 50 of the *trp*+ *str* recombinants were also tested for $C^{14}$-TdR incorporation.
§ See footnote to Table 2.

TABLE 4

*Transduction of the tdk+ gene by phage P1*kc *from strain KY484 to KY688**

| Selective marker | Unselected marker | | | Number of transductants |
|---|---|---|---|---|
| | *ton* | *trp* | *cys* | |
| *tdk+* | 1† | 1 | 1 | 137 |
| | 1 | 1 | 0 | 175 |
| | 1 | 0 | 0 | 64 |
| | 0 | 1 | 0 | 16 |
| | 0 | 0 | 1 | 1 |
| | 0 | 0 | 0 | 107 |
| | | | Total | 500 |

* Donor (KY484): *tdk+ tonB+ trpE+ cysB*
Recipient (KY688): *tdk-1 tonB trpE cysB+*
† 1 represents markers from the donor and 0 represents markers from the recipient.

It was then attempted to determine the position of the *tdk* locus relative to the uridine diphosphoglucose pyrophosphorylase (*UDPG*) gene (SHAPIRO 1965) and *att80* (attachment site for prophage $\phi$80). Two transduction experiments were performed with phage P1*kc* (Table 5). The result of Experiment 1 shows that the *tdk* gene is linked to *tonB* more closely than is *UDPG*. The result of Experiment 2 shows that the presence of prophage $\phi$80 on the chromosome strikingly reduces the linkage values for the *tonB-tdk* and *tonB-UDPG* pairs but not for the *trp-tonB* pair. These results strongly suggest that *att80* is located

TABLE 5

*Mapping of the* tdk *gene relative to the* UDPG *gene by transduction*

| Selective marker | Unselected marker | | | | Number of transductants | |
|---|---|---|---|---|---|---|
| | *UDPG* | *tdk* | *ton* | *trp* | Exp. 1a | Exp. 2 |
| *trp+* | 1* | 1 | 1 | . | 41 | 0 |
| | 0 | 1 | 1 | . | 10 | 0 |
| | 0 | 0 | 1 | . | 38 | 82 |
| | 0 | 1 | 0 | . | 4 | 0 |
| | 0 | 0 | 0 | . | 107 | 118 |
| | | | | Total | 200 | 200 |
| | | | | | Exp. 1b | |
| *tdk+* | 1 | . | 1 | 1 | 118 | ... |
| | 1 | . | 1 | 0 | 26 | ... |
| | 1 | . | 0 | 0 | 44 | ... |
| | 0 | . | 0 | 0 | 2 | ... |
| | 0 | . | 1 | 1 | 8 | ... |
| | 1 | . | 0 | 1 | 2 | ... |
| | | | | Total | 200 | ... |

Experiment 1 Donor (W4597): *UDPG tdk+ tonB+ trpE+*
Recipient (KY688): *UDPG+ tdk-1 tonB trpE*
Experiment 2 Donor (KY976): *UDPG tdk+ tonB+ trpE+* ($\phi$80)
Recipient (KY980): *UDPG+ tdk-1 tonB trpE* ($\phi$80)
* See footnote to Table 4.

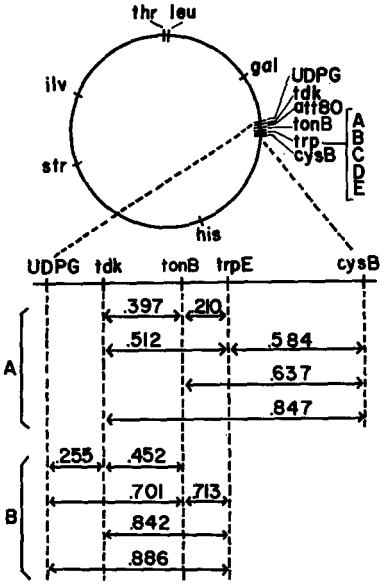


FIGURE 1.—Genetic map of the *tdk* region of the *E. coli* chromosome. A and B represent the map distances calculated from the transduction data presented in Tables 4 and 5 (Experiment 1a), respectively. The relative distances (d) between pairs of markers are represented as $d = 1 - r$, where $r = 11/(11 + 10 + 01)$ or $r = 11/(11 + 10 + 10)$ (1 represents markers from the donor, and 0 represents markers from the recipient).

between *tonB* and *tdk*. The map distances between the markers have been calculated from the results of Tables 4 and 5 (Experiment 1a) according to NESTER, SCHAFER and LEDERBERG (1963) and are shown in Figure 1.

In agreement with these results, 17 independently isolated mutants carrying deletions at the *tonB-trp* region all showed the Tdk$^+$ phenotype. Moreover, when an episome F'*trp* (FREDERICQ 1964) carrying the *tonB*$^+$, *trp*$^+$ and *cysB*$^+$ genes was transferred to F$^-$ *tdk-1* mutant cells, the F' strain obtained (KY894) still showed the Tdk$^-$ phenotype, suggesting that the F'*trp* episome does not carry the *tdk*$^+$ gene. It has been shown that an intact *att80* region is present on this episome (NAGATA personal communication; FRANKLIN cited in SIGNER and BECKWITH 1966).

*Specialized transduction of the* tdk *gene with phage φ80:* As was expected from the chromosomal location of *tdk*, a phage lysate of *tdk*$^+$ bacteria lysogenic for φ80 was found to transduce the *tdk*$^+$ gene to *tdk-1* mutant bacteria (nonlysogenic strain KY895). The frequency of transduction was approximately $1.4 \times 10^{-7}$ per plaque forming unit under the conditions used. Fifty transductant colonies were picked, purified and their properties examined. Sixteen of them produced high frequency transducing (HFT) lysates upon induction with mitomycin C (1 μg/ml), and spontaneously segregated *tdk* mutant cells at relatively high frequency. These results suggest that they are heterogenotes carrying both *tdk-1* and *tdk*$^+$ alleles: The HFT lysates could transduce the *tdk*$^+$ gene at a frequency of about $3 \times 10^{-2}$ per plaque forming unit when the recipient was lysogenic for φ80. If abortive transductants occurring at about ten times higher frequency are included, the ratio of transducing particles to plaque formers becomes $3 \times 10^{-1}$ which is an order of magnitude higher than the value usually found with HFT lysates from a heterogenote carrying φ80 and φ80d*t* (defective, tryptophan transducing φ80 phage, MATSUSHIRO 1963).

The fact that the heterogenote (*tdk-1/tdk*$^+$) is phenotypically Tdk$^+$ suggests that *tdk*$^+$ is dominant over the *tdk-1* allele. As was expected from the position of the *tdk* gene on the chromosome, the HFT lysates from the heterogenotes did not transduce the *trpA*$^+$ marker to *trpA* bacteria at high frequency, and conversely, phage $\phi$80d$t_0$ or $\phi$80p$t_1$ carrying *trp*$^+$ markers failed to transduce the *tdk*$^+$ marker to *tdk-1* bacteria at high frequency. Also, no evidence was obtained suggesting that the former HFT lysates transduce *UDPG*$^+$ or *supC*$^+$ to their respective mutant bacteria.

The HFT lysate from one of the heterogenotes (KY904) was then examined by CsCl equilibrium density gradient centrifugation to determine the buoyant density of *tdk*$^+$-transducing phage as compared to that of normal phage $\phi$80. As seen in Figure 2, the *tdk*$^+$ transducing particles formed a peak which is distinct from that of normal $\phi$80 also present in the HFT lysate. The density of the transducing particles is about 1.489 g cm$^{-3}$, when calculated from the published data for $\lambda$ (1.508 g cm$^{-3}$) (WEIGLE, MESELSON and PAIGEN 1959) and $\lambda b_2$ (1.491 g cm$^{-3}$) (KELLENBERGER, ZICHICHI and WEIGLE 1960). The density of $\phi$80 obtained (1.494 g cm$^{-3}$) also coincided closely with the values reported by the previous workers (MATSUSHIRO, SATO and KIDA 1964; OZEKI personal communication). Furthermore, when transducing particles were assayed using a nonlysogen (KY982) instead of a $\phi$80 lysogen as the recipient, transduction occurred at ten times higher frequency if excess normal $\phi$80 phage was also added to the transduction mixtures. All these results point to the conclusion that the transducing

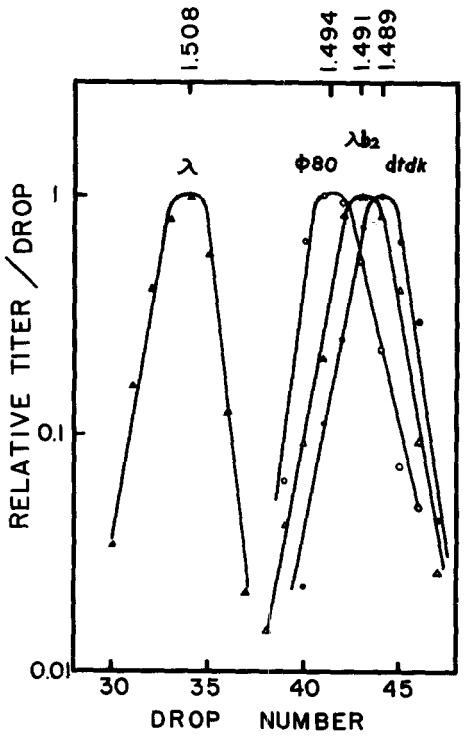


FIGURE 2.—Distribution of phage $\phi$80 and *tdk*$^+$ transducing particles, $\phi$80d*tdk*$^+$, in a CsCl density gradient. To the appropriately diluted HFT lysate from strain KY904, CsCl was added to a final density of 1.50 g cm$^{-3}$ and this was spun at 20°C for 24 hours at 23,500 rpm in an SW-39 rotor of a Spinco model L ultracentrifuge. After centrifugation, the bottom of the tube was pierced and drops were collected as described by WEIGLE, MESELSON and PAIGEN (1959). Plaque-forming phage titers for each tube were determined employing strain KY123 or KY124 as indicator for $\phi$80 or $\lambda$ (and $\lambda b_2$), respectively. For determination of transducing phage, cells of strain KY981 infected with an appropriate dilution of each fraction were plated on FU medium and transductants scored after 2 days incubation at 37°C. Phage $\lambda$ and $\lambda b_2$ served as density references.

phage are defective, and therefore will be referred to as $\phi$80d*tdk*$^+$ (defective *tdk*$^+$-transducing phage).

When *tdk-1* mutant cells are infected with the HFT lysate, activity of TdR kinase appeared immediately, and incorporation of $C^{14}$-TdR into the acid-insoluble fraction followed after 30 minutes (Figure 3a). If the same *tdk-1* mutant but lysogenic for $\phi$80 was infected, expression of the *tdk*$^+$ gene as judged by the TdR kinase activity was limited to the lower level at least at the initial stage, and the $C^{14}$-TdR incorporation was also reduced accordingly (Figure 3b). When cells of the heterogenote were treated with mitomycin C (1 $\mu$g/ml), specific activity of the enzyme increased about 8-fold after 90 min incubation at 37°C (Figure 4). No appreciable increase of specific activity was observed, however, when nonlysogenic or $\phi$80 lysogenic *tdk*$^+$ bacteria were similarly treated with mitomycin C. It was also shown by enzyme mixing experiments that this increase of the enzyme activity is not due to the presence of an activator in the extracts from induced bacteria or to the presence of an inhibitor in the extracts from noninduced bacteria. The enzyme activity in extracts from the induced heterogenote was also inhibited by dTTP and was resistant to heat treatment (70°C for 5 min) as in the case of wild-type enzyme from noninduced bacteria (OKAZAKI and KORNBERG 1964).

## DISCUSSION

The genetic data presented above establish that the genes studied so far in the *tdk-trp* region of the *E. coli* chromosome are located in the order *UDPG-tdk-att80-tonB-trp-cysB*. Relative positions of *UDPG*, *att80*, *tonB*, *trp* and *cysB* genes found in the present study are in agreement with the orders previously published, *supC-att80-tonB-trp-cysB* (SIGNER, BECKWITH and BRENNER 1965) and *supC-UDPG-att80-trp* (SHAPIRO 1966).

Cotransduction frequencies of the marker pairs spanning *att80*, i.e. *trp-tdk*, *trp-UDPG*, *tonB-tdk* and *tonB-UDPG*, are greatly reduced when prophage $\phi$80 is present in both donor and recipient bacteria used. These results confirm the recent finding of SIGNER (1966) that $\phi$80 lysogeny increases the apparent distance between marker pairs spanning the prophage, and support the model of linear insertion of prophage into the bacterial chromosome (CAMPBELL 1962; FRANKLIN, DOVE and YANOFSKY 1965; ROTHMAN 1965; SIGNER 1966). In addition, it became clear from the present study that *att80* lies between the *tonB* and *tdk* markers on the chromosome.

MATSUSHIRO (1963) has shown that genes of the tryptophan operon can be specifically transduced by $\phi$80, and SIGNER (1966) extended this observation to include several other genes located on either side of the prophage $\phi$80. It has also been found that $\phi$80d*supC*$^+$ carries *UDPG*$^+$, whereas $\phi$80-d*UDPG*$^+$ does not carry *supC*$^+$ (ECHOLS, cited in SHAPIRO 1966). The present findings that $\phi$80d*tdk*$^+$ particles do not carry *trp*$^+$, *UDPG*$^+$ or *supC*$^+$ and that $\phi$80d$t_0$ or $\phi$80p$t_1$ similarly does not carry *tdk*$^+$ markers are all consistent with the order *supC-UDPG-tdk-att80-tonB-trp-cysB*. Previous studies of MATSUSHIRO (1963) and SIGNER (1966) revealed that transducing phage that carries a given bacte-

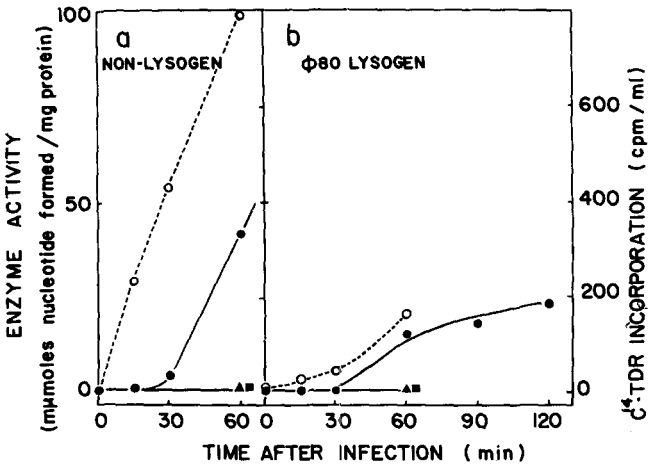


FIGURE 3.—$C^{14}$-TdR incorporation and TdR kinase activity in *tdk-1* bacteria after infection with an HFT lysate from strain KY904. (a) Nonlysogenic strain (KY895); (b) φ80 lysogenic strain (KY896). Log-phase bacteria suspended in T1 buffer were infected with phage at a multiplicity of 5 and incubated at 37°C for 10 min. After dilution with a prewarmed glucose-minimal medium supplemented with 0.2% Casamino acids and 20 μg L-tryptophan per ml, the culture was divided into two parts. One part was incubated at 37°C with shaking, aliquots removed at intervals, and the cells harvested were used for enzyme assay. Another part was used to determine incorporation of $C^{14}$-TdR into the acid-insoluble fraction by the procedures described under MATERIALS AND METHODS. For enzyme assay, the reaction mixtures with crude extract (400 μg protein) were incubated for 60 min at 37°C. Enzyme activity (○) or $C^{14}$-TdR incorporation (●) after infection with an HFT lysate. Noninfected cells (▲) and cells infected with normal φ80 (■) were taken as controls in $C^{14}$-TdR incorporation.

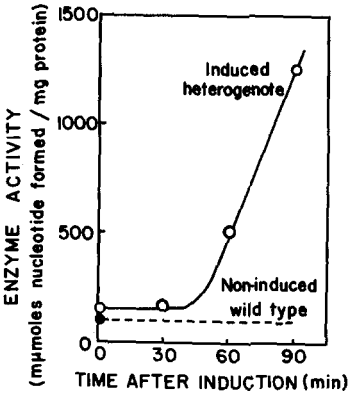


FIGURE 4.—Increase of TdR kinase activity during induction of a heterogenote (KY904) with mitomycin C. To a log-phase culture in a peptone-glucose medium was added mitomycin C (1 μg/ml) at time 0, and samples were taken thereafter in several intervals. Reaction mixtures with crude extract (20 μg protein) were incubated at 37°C for 60 min. Heterogenote (○) and noninduced wild-type cells (●).

rial marker also carries the markers located between it and the prophage but not the markers on the other side of *att80*. It would be expected then that both φ80d*supC*$^+$ and φ80d*UDPG*$^+$ carry the *tdk*$^+$ gene.

The *tdk-1* mutant having an episome F′*trp* which is known to carry *tonB*$^+$, *trp*$^+$, *cysB*$^+$ *colB* and *colV* (FREDERICQ 1964) and *att80* (NAGATA personal communication; FRANKLIN, cited in SIGNER and BECKWITH 1966) showed the Tdk$^-$ phenotype. Two possibilities were considered to explain this result: (1) The episome does not carry the *tdk*$^+$ gene. (2) The episome carries the *tdk*$^+$ gene, but the *tdk-1* mutant allele is dominant over *tdk*$^+$. The second possibility seems

to be excluded, however, since the transductional heterogenotes are phenotypically Tdk$^+$, that is, the *tdk-1* allele is recessive to *tdk*$^+$. It is therefore concluded that the *tdk*$^+$ gene is not present on the F'*trp* episome.

The synthesis of TdR kinase in nonlysogenic and $\phi$80 lysogenic *tdk-1* mutant bacteria upon infection with HFT lysates may also deserve some comment. The fact that the TdR kinase activity appears without appreciable lag even in the case of the $\phi$80 lysogen suggests that expression of the *tdk*$^+$ gene on $\phi$80d*tdk*$^+$ particles is not specifically repressed by a phage immunity substance, though the reduced activity found with the lysogen compared to the nonlysogen might be a result of more general or indirect effect of the phage immunity. At present, nothing is known about regulation of TdR kinase synthesis. If an operator-repressor type of control also applies to the present system of the *tdk* gene, these results might be interpreted to mean that the $\phi$80d*tdk*$^+$ genome contains the operator region for the TdR kinase operon as in the case of $\phi$80p$t_0$ and the *trp* operon studied by SATO and MATSUSHIRO (1965).

There are several possibilities as to the nature of the *tdk-1* mutation. The possibility that the *tdk-1* mutant involves a regulator gene and produces a super-repressor, as does mutant $i^s$ of the lactose operon (JACOB and MONOD 1961), seems to be ruled out, since the *tdk-1* allele is recessive to *tdk*$^+$ (Figure 4). In view of the rapid synthesis of TdR kinase in *tdk-1* mutant bacteria after infection with an HFT lysate (Figure 3a) or in the heterogenotic cells after induction with mitomycin C (Figure 4), it seems most likely that *tdk* represents the structural gene for TdR kinase. In support of this conclusion, several additional *tdk* mutants, including temperature sensitive ones, have been isolated recently. Preliminary evidence suggests that these mutations all occurred at or around the *tdk* gene on the chromosome, and that partial complementation takes place between some of these mutant alleles when combined in heterogenotes. Further characterization of these mutants is in progress.

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## SUMMARY

The *tdk-1* mutant, which lacks the activity of deoxythymidine (TdR) kinase, is closely linked to the cluster of genes controlling tryptophan biosynthesis. The results of crosses through mating or transduction with phage P1*kc* showed that the six genes studied are located on the *E. coli* chromosome in the order *UDPG-tdk-att80-tonB-trp-cysB*.—Phage $\phi$80 transduces the *tdk*$^+$ marker to *tdk-1* mutant bacteria at a low frequency. Among the transductants obtained, heterogenotes carrying both $\phi$80 and a defective transducing phage named $\phi$80d*tdk*$^+$, were isolated. Such heterogenotes (*tdk-1/tdk*$^+$) are phenotypically Tdk$^+$ indicating that the *tdk-1* mutant allele is recessive. A high-frequency transducing (HFT) lysate was obtained upon induction of such heterogenotic bacteria. The buoyant density of $\phi$80d*tdk*$^+$ particles differs from that of normal $\phi$80.—When *tdk-1*

mutant bacteria were infected with an HFT lysate, the activity of TdR kinase appeared immediately. Also, the enzyme activity in the heterogenotic cells increased markedly upon induction with mitomycin C. Evidence suggests that *tdk* represents the structural gene for TdR kinase.

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