The Induction of Deoxythymidine Kinase by Bacteriophage T4

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SUMMARY

A mutant of Escherichia coli, tdk 32, defective for deoxythymidine kinase (ATP: thymidine 5'-phosphotransferase EC 2.7.1.21) and therefore unable to incorporate thymidine into DNA has been used to study alterations in thymidine utilization resulting from phage infection. Infection by the T-even phages leads to the rapid incorporation of [3H]-thymidine into DNA which results from the induction of deoxythymidine kinase activity as first reported by Hiraga, Igarashi & Yura (1967). No effect was observed following infection of tdk 32 by phages T3, T7, λ, Pl and Mu-1. The T4-induced enzyme differs from the wild-type cell enzyme in several properties including heat stability, deoxycytidine triphosphate stimulation and pH optimum and the ability to induce deoxythymidine kinase can be lost by mutation in a T4 gene (tk) linked to rI. T4tk mutant-infected tdk 32 cells fail to incorporate [3H]-thymidine or 5-bromodeoxyuridine into DNA and the latter property formed the basis for the selection of the phage mutants. The T4tk gene is non-essential for growth both in wild type and tdk mutant cells since deoxythymidine monophosphate can be synthesised by the de novo pathway.

INTRODUCTION

The utilization of thymidine as a precursor of DNA in Escherichia coli requires the presence of an active deoxythymidine kinase (Okazaki & Kornberg, 1964a). When the endogenous synthesis of deoxythymidine monophosphate is blocked with 5-fluorouracil the formation of this precursor, and therefore cell growth, becomes dependent on an exogenous supply of thymidine (Igarashi, Hiraga & Yura, 1967). Deoxythymidine kinase will also use 5-bromodeoxyuridine as a substrate for incorporation into DNA and is therefore also of importance in studies using this thymidine analogue (Okazaki & Kornberg, 1964a).

Mutants of Escherichia coli lacking an active deoxythymidine kinase (tdk mutants) have been isolated and used to study the utilization of exogenous thymidine following infection with phage (Hiraga et al. 1967). Hiraga et al. (1967) showed that the ability of tdk mutant bacteria to incorporate thymidine into DNA was restored soon after infection by phages T2 and T4 but not by T1 and T5. The deoxythymidine kinase activity induced by T-even phage infection was more heat labile than the wild type cell enzyme suggesting that the phage-induced activity was qualitatively different from the bacterial enzyme.

In this paper deoxythymidine kinase-negative mutants of Escherichia coli have been used in further studies of the uptake of thymidine by phage-infected cells and of the properties of the phage T4-induced deoxythymidine kinase. It will be shown that the tdk cell mutants can be used to select deoxythymidine kinase negative mutants of phage T4. Apart
from showing that the T4-induced deoxythymidine kinase is coded for by a phage gene, this system also provides a means to suppress completely the incorporation of thymidine into T-even phage DNA.

During the preparation of this paper Chace & Hall (1973) reported the isolation and some properties of deoxythymidine kinase-negative mutants of phage T4 with results very similar to those presented below.

METHODS

Bacteria and bacteriophage. All bacteria employed were strains of Escherichia coli. Strain W3110, wild-type for deoxythymidine kinase (tdk⁺), was the parent strain used for isolation of the tdk mutants. Strain B, non-permissive for amber mutants, and the permissive strain CR63, were used as host and indicator strains for genetic crosses with the phage T4D mutants. Phages λv, P1kc and Mu-I were propagated on strain W3110, the T4D amber mutants on strain CR63 and all other T-phages on strain B. The T4D rI, rII and rIII mutants were isolated as spontaneous mutants and identified by the criteria of Benzer (1957).

Isolation of Escherichia coli tdk mutants. Mutants unable to synthesise deoxythymidine kinase (tdk mutants) were selected by a method suggested by Hiraga et al. (1967) as a result of studies with their spontaneously isolated tdkI mutant. An overnight culture of W3110, diluted 50-fold in ETCG medium (MacHattie et al. 1967) containing 100 µg/ml 5-bromodeoxyuridine (Calbiochem Ltd.) was grown to saturation at 37 °C, diluted ten-fold in phosphate buffer, pH 7 (Hershey & Chase, 1952), and irradiated with visible light. About 0.5 % of the cells survived a 2.5 h exposure compared with 60 % of control cells grown in medium containing 100 µg/ml thymidine (Sigma, London) in place of 5-bromodeoxyuridine. The surviving cells were exposed to two further selection cycles of growth in 5-bromodeoxyuridine medium followed by visible light irradiation. Samples of the survivors of the third cycle of irradiation were plated at low density on nutrient agar plates and the single colonies replicated to FU medium plates (nutrient agar containing 25 µg/ml 5-fluorouracil (Sigma, London), 25 µg/ml uridine (Calbiochem Ltd.) and 50 µg/ml thymidine) which by blocking the de novo pathway of deoxythymidine monophosphate synthesis selectively inhibits growth of tdk mutant cells (Igarashi et al. 1967). Twelve colonies of about 200 screened, grew poorly on FU plates and upon retesting were confirmed as being sensitive to FU medium. These mutant clones were considered as being putative tdk mutants.

Incorporation of [3H]-thymidine into DNA. Cell cultures were grown with aeration at 30 °C in minimal medium, either M9 medium (Adams, 1959) supplemented with 1 mg/ml Difco casamino acids (M9C) or ETCG medium. At 2 x 10⁸ cells/ml the medium was supplemented with 250 µg/ml 2′-deoxyadenosine (Sigma, London), 2.5 µg/ml thymidine and 1–2 µCi/ml [3H]-thymidine (Radiochemical Centre, Amersham). 50 or 100 µl samples were removed at intervals, applied to 2.5 cm squares of Whatman 3 M paper each of which was washed twice for 15 min in 10 % (w/v) ice-cold trichloracetic acid and rinsed in water. The dried paper squares were suspended in 10 ml toluene-based scintillant (Ritchie & Malcolm, 1970) and the radioactivity counted in a scintillation counter. For phage-infected cultures the medium was supplemented with 20 µg/ml L-tryptophane and the cells were infected at a multiplicity of 5 pfu/cell, usually immediately after addition of the [3H]-thymidine.

Incorporation of 5-bromodeoxyuridine into phage T4D DNA. An overnight culture of tdk 32 was diluted 50-fold in M9C medium containing 20 µg/ml L-tryptophane and incubated with aeration at 37 °C. At a titre of 1 x 10⁸ cells/ml the medium was supplemented
with 250 μg/ml 2'-deoxyadenosine and one generation later with 100 μg/ml 5-bromodeoxyuridine. The cells were immediately infected with phage T4D+ at an input multiplicity of 0.1 p.f.u./cell and incubated for 1 h when lysis was induced by bubbling with chloroform. For control lysates the 5-bromodeoxyuridine was replaced by 100 μg/ml thymidine.

**Selection of T4D tk mutants.** A modification of the method used for isolating the *Escherichia coli* tdk mutants and also based on the increased visible light sensitivity of 5-bromodeoxyuridine labelled DNA was used to attempt the isolation of deoxythymidine kinase negative mutants from phage T4D. Since the synthesis of deoxythymidine kinase is induced following infection of tdk 32 cells by T4D+ phage the resulting phage progeny will contain 5-bromodeoxyuridine, if present in the growth medium, and will consequently be sensitive to inactivation by visible light (Stahl et al. 1961). However, the same cells infected by a deoxythymidine kinase-negative mutant of T4 will not incorporate 5-bromodeoxyuridine and the progeny phage, being relatively resistant to visible light inactivation, will be at a selective advantage and their frequency, relative to T4D+ will be enhanced among the survivors of visible light irradiation. During growth of the phage in 5-bromodeoxyuridine medium it is essential that conditions of single infection are used to prevent loss of potential mutants by coinfection with wild-type phage.

A T4D+ phage stock grown on tdk 32 cells for one generation in 5-bromodeoxyuridine medium, as described above, was exposed to visible light for 3 h and the survivors (~ 5 %) were regrown to a high titre stock in the absence of 5-bromodeoxyuridine. This selection treatment was repeated three more times.

**Enzyme extraction.** Two hundred ml batches of cells were grown in H-broth (Krieg, 1959) with aeration at 37 °C to 2 × 10^8 cells/ml when the cells were harvested either immediately (uninfected cell extracts) or after 15 min incubation following the addition of phage (input multiplicity of 5 p.f.u./cell) for infected cell extracts. After chilling in ice the cells were collected by sedimentation (2500 g for 15 min at 20 °C), washed twice in ice-cold 0.9 % saline, once in 10 mm-tris buffer, pH 7 containing 5 mm-mercaptoethanol and 5 μM-thymidine, suspended in 10 ml of the same buffer and sonicated in an ice bath with a probe sonicator (Dawe Soniprobe). Bovine serum albumin (2.0 mg/ml) was added to the disrupted cells suspension and after sedimentation at 100000 g for 1 h at 4 °C the supernatant fraction containing the thymidine kinase activity (crude extract) was collected and stored at ~ -20 °C.

**Enzyme assay.** Deoxythymidine kinase activity was measured by the conversion of [3H]-thymidine to thymidine nucleotides which can be separated from the deoxyribonucleoside substrate and from the base thymine, which is also produced due to the activity of deoxythymidine phosphorylase. Two separation methods were used: (a) specific adsorption of thymidine nucleotides to Whatman DE 81 paper (Bresnick & Thompson, 1965; Sheinin, 1966) and (b) paper chromatography.

The standard reaction mixture contained in 100 μl: 50 μl crude extract (100 μg of protein), 0.1 mm-[3H]-thymidine (equivalent to 10 μCi), 7 mm-adenosine triphosphate, 7 mm-MgCl₂, 2.5 mm-MnCl₂ and 0.02 mm-phosphate buffer at pH 7.5 for bacterial enzyme assays or pH 7.0 for phage enzyme assays. Reaction mixtures were incubated at 37 °C and chilled in ice to stop the reaction. For the standard assay 10 μl samples were applied to 2 cm diam. discs of Whatman DE 81 paper, washed, for 7 min at 37 °C, once in 4 mm-ammonium formate, pH 4, containing 5 μM-thymidine and once in 4 mm-ammonium formate, pH 4. After washing in distilled water the discs were rinsed in absolute alcohol, dried, and the radioactivity counted as described above.

Where necessary thymidine and its phosphorylated products, deoxythymidine mono-,
Fig. 1. Effect of phage T4 infection on the incorporation of [3H]-thymidine by wild type (W3110) and mutant (tdk 32) bacteria. Cells were grown in ETCG medium containing 20 μg/ml L-tryptophane at 30 °C with aeration. At 2 × 10⁶ cells/ml the medium was supplemented with 250 μg/ml 2'-deoxyadenosine, 2.5 μg/ml thymidine and 1 μCi/ml [3H]-thymidine (t = 0 min). At t = 20 min (↓) one half of each culture was infected with 5 p.f.u./cell of T4B⁺ phage. 100 μl samples were removed at intervals and assayed for acid-insoluble radioactivity. ■ ■, W3110; ___, tdk 32; ● ●●, T4-infected tdk 32.

di- and triphosphate, were separated from each other by descending chromatography for 16 h at room temperature on Whatman no. 1 paper in glacial acetic acid: butanol: water solvent in a v/v ratio of 1:2:1. The dried chromatograms were cut into 1 cm strips transverse to the direction of solvent flow and the strips counted for radioactivity.

Visible-light irradiation. Ten ml samples of phage and bacteria were suspended in phosphate buffer and irradiated in closed 90 mm plastic Petri dishes at a distance of 15 cm from a 40 W Philips Actina Blue fluorescent tubular lamp.

Genetic mapping of T4D tdk 26. Crosses were carried out at 37 °C in tryptone broth (Mahmood & Lunt, 1972) using standard procedures. The tdk genotypes were scored on the progeny from single plaques using a simplified assay for the incorporation of [3H]-thymidine into DNA. Whole fresh plaques, removed as agar plugs from plates with a Pasteur pipette, were suspended in 2 ml M9C medium and 0.5 ml was added, together with 250 μg/ml 2'-deoxyadenosine, 2.5 μg/ml thymidine and 10 μCi/ml [3H]-thymidine, to an equal vol. of tdk 32 cells grown to 2 × 10⁸ cells/ml in M9C medium containing 20 μg/ml L-tryptophane. The infected cultures were incubated at 30 °C for 90 min when 100 μl samples were removed and assayed for acid-insoluble radioactivity. With this method the
Table 1. Deoxythymidine kinase activity in crude extracts from uninfected and T4-infected cells*

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Wild type (W3110)</th>
<th>Mutant (tdk 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>26</td>
<td>0.7</td>
</tr>
<tr>
<td>T4D+</td>
<td>55</td>
<td>44</td>
</tr>
<tr>
<td>T4D tk 26</td>
<td>19</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* The reaction products from a 20 min incubation of a standard reaction mixture (see Methods) were separated by paper chromatography and the counts in the peaks of deoxythymidine mono-, di- and triphosphates were summed and expressed as a percentage of the total [H]-thymidine in the reaction mixture. Since the synthesis of the di- and tri-phosphate depends on deoxythymidine monophosphate formation, these values represent the percentage of [H]-thymidine phosphorylated to the monophosphate.

tk+ samples incorporated 3000 to 10000 ct/min and tk phage 50 to 200 ct/min above background.

RESULTS

Characterization of Escherichia coli tdk mutants

The mutant clones sensitive to growth in FU medium showed normal growth rates at 30 and 37 °C in broth and synthetic media and produced colonies on nutrient agar indistinguishable from wild type. Like the original tdk 1 mutant (Hiraga et al. 1967) they were also resistant to phage T1. However, unlike wild type, the mutant cells failed to incorporate appreciable amounts of [H]-thymidine into acid-insoluble material (Fig. 1) and were resistant to visible light after growth in 5-bromodeoxyuridine medium. Crude extracts of tdk 32, the mutant used in further studies, were able to phosphorylate thymidine to only about 1/40th the extent of tdk+ cell extracts (Table 1). These properties are consistent with those expected for mutants which fail to synthesize normal amounts of deoxythymidine kinase (Hiraga et al. 1967).

Effect of phage infection on thymidine utilization

Infection of wild-type or tdk 32 cells by phages T2H hr 1, T4B+, T4D+ and T6+ stimulated the incorporation of [H]-thymidine into acid-insoluble form. In the typical example shown for T4B+ the increased rate of [H]-thymidine incorporation was observed by 10 to 15 min after infection at 30 °C (Fig. 1). While the rate of incorporation was the same for both infected cultures the difference in rates between infected and uninfected cells was more pronounced for the tdk mutant cells.

Increased deoxythymidine kinase activity was detected in crude extracts of both W3110 and tdk 32 cells following infection with phage T4D+ (Table 1) and also for T2H hr 1. By contrast, infection of tdk 32 by phages T3+, T7+ λv, PlkC and Mu-1 failed to stimulate [H]-thymidine incorporation and gave results identical to the uninfected cells.

Properties of Escherichia coli and T4-induced deoxythymidine kinases

The deoxythymidine kinase activities of crude extracts of W3110 (bacterial enzyme) and of tdk 32 infected with T4D+ (phage enzyme) were compared under a variety of conditions.

Incubation of the bacterial enzyme at 45 °C caused no appreciable loss of activity during
Fig. 2. Heat stability of bacterial and phage T4-induced thymidine kinase. Crude extracts from wild type (W3110) bacteria (○—○) and from T4D+ infected thymidine kinase-negative (tdk 32) bacteria (■—■) were incubated at 45 °C and 50 μl samples were removed at intervals, incubated for 10 min in a standard reaction mixture and assayed for deoxythymidine kinase activity as described in Methods. Relative activities are calculated as a percentage of the unheated sample.

a 20 min incubation period, the phage enzyme, however, was heat labile and after 20 min incubation only 25% of the original activity remained (Fig. 2).

The activities of both enzymes varied with the pH of the reaction mixture. The bacterial enzyme, as previously reported (Okazaki & Kornberg, 1964a), had an optimum activity at pH 7.5, whereas for the phage enzyme the pH optimum was lower at pH 7.0 (Fig. 3). This difference, although relatively small, was consistently observed.

Pyrimidine di- and tri-phosphates are known to affect the activity of the bacterial enzyme, deoxythymidine triphosphate is a competitive inhibitor whereas deoxycytidine triphosphate stimulates the activity (Okazaki & Kornberg, 1964b). The activity of the phage enzyme was insensitive to deoxycytidine triphosphate and the inhibitory effect of deoxythymidine triphosphate was only about 50% of that of the bacterial enzyme over the range of concentrations tested (Fig. 4).

The reaction rates of both enzymes were measured at varying substrate concentrations. From the Lineweaver-Burk reciprocal plot the $K_m$ values were calculated as $3.3 \pm 0.2 \times 10^{-5}$ M-thymidine for the bacterial enzyme and $1.0 \pm 0.3 \times 10^{-5}$ M-thymidine for the phage enzyme when measured under similar conditions. The slope of the plot for the phage enzyme was about fourfold greater than that for the bacterial enzyme. Therefore, although the $K_m$ values are in the same range it is worth noting that the phage enzyme has a faster reaction rate.
Isolation of deoxythymidine kinase-negative mutants of phage T4D

After exposure of a T4D+ phage stock to four cycles of growth in 5-bromodeoxyuridine medium followed by visible-light irradiation, the entire phage population had become almost totally resistant to the 5-bromodeoxyuridine-induced light-sensitive reaction. Several phage stocks derived from individual plaques picked from the final lysate were tested for their visible-light resistance after growth in 5-bromodeoxyuridine medium and were found to be as resistant to inactivation as phages grown in medium with thymidine in place of 5-bromodeoxyuridine (Fig. 5).

Fig. 3. Effect of pH on the deoxythymidine kinase activity of crude extracts from wild type (W3110) bacteria (○ —○) and from phage T4D+-infected thymidine kinase-negative (tdk 32) bacteria (● —●). Enzyme activity was determined following a 10 min incubation in a standard reaction mixture as described (see Methods) except that the pH of the 0.02 M-phosphate buffer in the reaction mixture was varied. Activity is expressed relative to that of the respective pH optima, i.e. pH 7 for the phage enzyme and pH 7.5 for the bacterial enzyme.

Fig. 4. Effect of deoxypyrimidine triphosphate concentration on the deoxythymidine kinase activity of crude extracts from wild type (W3110) bacteria and from phage T4D+-infected deoxythymidine kinase-negative (tdk 32) bacteria. Standard reaction mixtures were incubated for 10 min in the presence of either deoxycytidine triphosphate (dCTP) or deoxythymidine triphosphate (dTTP) at the concentrations indicated and assayed as described in Methods. Enzyme activities are expressed as a percentage of the control reaction mixtures without added triphosphate [●—●, W3110+dCTP; ——]}, W3110+dTTP; ○—○, T4-infected tdk 32+dCTP; —•—•, T4-infected tdk 32+dTTP.

Isolation of deoxythymidine kinase-negative mutants of phage T4D

After exposure of a T4D+ phage stock to four cycles of growth in 5-bromodeoxyuridine medium followed by visible-light irradiation, the entire phage population had become almost totally resistant to the 5-bromodeoxyuridine-induced light-sensitive reaction. Several phage stocks derived from individual plaques picked from the final lysate were tested for their visible-light resistance after growth in 5-bromodeoxyuridine medium and were found to be as resistant to inactivation as phages grown in medium with thymidine in place of 5-bromodeoxyuridine (Fig. 5).
Fig. 5. Visible-light inactivation of phage T4D+ and a visible-light-resistant mutant (T4D tk 26) grown in 5-bromodeoxyuridine medium. Phage stocks were grown in medium containing 5-bromodeoxyuridine (BUDR) or thymidine (TdR), diluted to $1 \times 10^6$ p.f.u./ml in phosphate buffer and assayed for surviving infectivity following exposure to several doses of visible light (see Methods for details). • •, T4D++BUdR; ■ ■, T4D++TdR; ○ ○, T4D tk 26+BUdR; □ □, T4D tk 26+TdR.

Cultures of tk 32 cells infected with the visible-light-resistant mutants were unable to utilize exogenously supplied thymidine as shown by failure of the mutant phage to stimulate the incorporation of [3H]-thymidine into DNA (Fig. 6). In addition the increased deoxythymidine kinase activity detected in crude extracts of W3110 and tk 32 cells after infection with phage T4D+ was not observed following infection with the mutant phage (Table 1).

From this evidence we conclude that the visible-light-selected T4 mutants (now referred to as tk mutants) have lost the ability to induce the synthesis of deoxythymidine kinase upon infection of Escherichia coli.

The T4 tk mutants exhibited normal plaque morphology, latent period and burst size when grown in either W3110 or tk 32 cells. No difference from wild-type phage was observed when stationary phase cells were used as host.

*Mapping the T4D tk mutation*

Before mapping the deoxythymidine kinase gene the original T4Dtk mutant isolate was backcrossed twice to tk+ phage to eliminate other possible mutations induced by repeated
T4-induced deoxythymidine kinase

Fig. 6. Incorporation of [H]-thymidine by deoxythymidine kinase-negative (tdk 32) bacteria infected with wild type (T4D\textsuperscript{+}) and a visible light-resistant mutant (T4D tk 26) phage. Experimental details as described for Fig. 1, except that phage was added at $t = 0$ min. ○—○, uninfected cells; ■—■, T4D\textsuperscript{+}-infected cells; ●—●, T4D tk 26-infected cells.

Table 2. Recombination between tk 26 and r mutants of T4D\textsuperscript{*}

<table>
<thead>
<tr>
<th>Cross</th>
<th>tk</th>
<th>r</th>
<th>Wild type</th>
<th>r tk</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. tk 26 × rI</td>
<td>45</td>
<td>44</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>47</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>33</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>2. tk 26 × rII</td>
<td>32</td>
<td>28</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>28</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>3. tk 26 × rIII</td>
<td>27</td>
<td>33</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>37</td>
<td>22</td>
<td>13</td>
</tr>
</tbody>
</table>

* For each cross approximately equal numbers of $r$ and $r^+$ plaques were isolated and their $tk$ genotype determined as described in Methods.

† Values given as percentage of recombinants to total progeny tested.

exposure to 5-bromodeoxyuridine during the selection process. Initially tk 26 was crossed with mutants in the rI, rII and rIII genes to determine the approximate map location. Analysis of all four genotypic products from these two-factor crosses revealed that tk 26 was linked to rI, giving a recombination frequency of about 8 %, but unlinked to rII and rIII (Table 2). To attempt a more precise location, tk 26 and the rI mutant were each crossed to amber mutants marking three genes close to rI: am BL292 (gene 55), am E727 (gene 49) and am H26 (gene e). These genes are arranged in the order 55–49–rI–e. All three
Table 3. Linkage of tk 26 to amber mutants proximal to rI*

<table>
<thead>
<tr>
<th>Cross</th>
<th>Titre/ml $\times 10^{-6}$†</th>
<th>Wild-type plaques‡</th>
<th>Recombination frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CR63</td>
<td>B</td>
<td>Total am+ plaques</td>
</tr>
<tr>
<td>1. tk 26 × am BL292</td>
<td>4.2</td>
<td>1.5</td>
<td>29/94</td>
</tr>
<tr>
<td></td>
<td>3.2</td>
<td>1.7</td>
<td>11/45</td>
</tr>
<tr>
<td>2. tk 26 × am E727</td>
<td>4.2</td>
<td>2.6</td>
<td>24/91</td>
</tr>
<tr>
<td></td>
<td>3.2</td>
<td>1.7</td>
<td>9/45</td>
</tr>
<tr>
<td>3. tk 26 × am H26</td>
<td>2.8</td>
<td>1.9</td>
<td>45/139</td>
</tr>
<tr>
<td>4. rI × am BL292</td>
<td>3.7</td>
<td>1.5</td>
<td>190/838</td>
</tr>
<tr>
<td></td>
<td>4.2</td>
<td>1.1</td>
<td>272/775</td>
</tr>
<tr>
<td>5. rI × am E727</td>
<td>2.9</td>
<td>1.3</td>
<td>194/749</td>
</tr>
<tr>
<td></td>
<td>3.6</td>
<td>1.1</td>
<td>292/745</td>
</tr>
<tr>
<td>6. rI × am H26</td>
<td>2.6</td>
<td>1.1</td>
<td>217/1027</td>
</tr>
<tr>
<td>7. am BL292 × am E727</td>
<td>4.6</td>
<td>0.40</td>
<td>—</td>
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<tr>
<td></td>
<td>3.2</td>
<td>0.26</td>
<td>—</td>
</tr>
</tbody>
</table>

* For crosses 1 to 6 the recombination frequency was calculated as the percentage of wild type (am+ tk+ or am+ r+) to total am+ plaques tested. The am+ plaques were selected on plates seeded with strain B and either picked and classified as tk or tk+, as described in Methods (crosses 1 to 3), or scored as rI or r+ by direct observation (crosses 4 to 6). For cross 7 the recombination frequency was calculated as twice the percentage of am+ to total plaques (from assay on strain CR63).

† Plaque titres in growth tube.
‡ Numbers of plaques classified.

amber mutants were rather loosely linked to both tk 26 and rI, giving recombination frequencies between 20 and 30% (Table 3). These long map distances make a precise mapping uncertain, however, they do suggest that tk 26 is more closely linked than rI to genes 55 and 49 and less linked to gene e, and indicate that the tk mutation is located between rI and gene 49. This order is substantiated by the result of the 3-factor cross: tk 26 rI × am H26. Of 191 r plaques picked from plates seeded with strain CR63, ten were tk+ and all of these recombinants were am+. The predominance of the unselected am+ allele among the selected tk+ rI recombinants is consistent with the rI gene being located between tk 26 and the e gene (am H26) to give the gene order 55–49–tk–rI–e.

DISCUSSION

The ability of *Escherichia coli* to utilize exogenous thymidine (or 5-bromodeoxyuridine) for DNA synthesis can be lost by mutation of the *tdk* gene which codes for the synthesis of deoxythymidine kinase (Hiraga *et al.* 1967). Under normal laboratory conditions the *tdk* mutant cells can depend entirely on the *de novo* pathway for deoxythymidine monophosphate synthesis, thus the *tdk* function is non-essential for DNA replication. Failure to incorporate 5-bromodeoxyuridine into DNA protects the *tdk* mutant cells from the 5-bromodeoxyuridine-induced visible light inactivation process observed with wild-type bacteria (Greer, 1960) and was the basis of the method used for selecting the *tdk* mutants used in this study.

By eliminating host-cell deoxythymidine kinase synthesis, the *tdk* mutants provide a suitable background for the study of phage-induced alterations in thymidine incorporation. The results of the present study together with those of Hiraga *et al.* (1967) reveal that of the phages examined, i.e. the seven T-phages and the unrelated temperate phages λ, P1 and Mu-1, only the T-even phages are able to stimulate the incorporation of exogenous thymidine upon infection of *tdk* bacteria.
**T4-induced deoxythymidine kinase**

The thymidine incorporation found with T-even-phage infected cells results from the induction of deoxythymidine kinase activity and the available evidence suggests that the phage-induced enzyme is qualitatively different from wild-type cell enzyme. Compared to the cell enzyme, the T4-induced enzyme shows two marked differences; it is inactivated at 45 °C and is not stimulated by deoxycytidine triphosphate. In addition, the T4-induced activity has a slightly lower pH optimum, a reduced inhibition by deoxythymidine triphosphate and, from the $K_m$ value, a somewhat increased reaction rate and substrate affinity. These differences would indicate that the T4-induced deoxythymidine kinase activity represents the appearance of a new enzyme rather than the induction of the cellular function. Since T4 DNA containing label from exogenously supplied $[^3H]$-thymidine is observed from the onset of T4 DNA synthesis it would appear that the T4 enzyme is induced prior to DNA synthesis and is an ‘early’ enzyme. These results confirm and extend those of Hiraga et al. (1967).

The *Escherichia coli* tdk mutants can also be used to select T4 phage mutants which fail to induce deoxythymidine kinase synthesis. Cultures of tdk bacteria infected with deoxythymidine kinase-negative (tk) T4 phage contain negligible amounts of deoxythymidine kinase and the incorporation of thymidine into DNA is completely suppressed, but nevertheless produce normal yields of phage with normal kinetics of synthesis.

The T4tk function provides a further example of a phage-coded enzyme concerned with thymidylate synthesis which duplicates a function present in wild-type cells and is non-essential under normal conditions. (The tk function is lethal when grown in cells in the presence of 5-fluorodeoxyuridine and uridine, in wild-type cells the inhibition can be overcome by the addition of thymidine.) T4 genes coding for thymidylate synthetase (Simon & Tessman, 1963), dihydrofolate reductase (Hall, 1967; Johnson & Hall, 1973) deoxycytidylate deaminase (Hall & Tessman, 1966) and nucleotide reductase (Yeh & Tessman, 1972) have been indentified. These enzymes are involved with the *de novo* synthesis of deoxythymidine monophosphate and the controlling genes map between genes 31 and 32 with the exception of the ribonucleotide reductase gene (nrdC) (Hall, Tessman & Karlström, 1967; Tessman & Greenberg, 1972). The mapping of the T4tk gene, which was undertaken to determine whether the tk function formed part of the ‘thymidylate’ cluster, shows clearly that it is unlinked to the thymidylate genes but is linked to the rI gene.

Our genetic analysis suggests that the tk gene is linked in the sequence 55-49-tk-rI-e. This order does not correspond precisely to the published sequence 49-nrdC-rI-tk-e, derived from a three-factor cross with the markers nrdC, rI and tk (Chace & Hall, 1973). While there is no doubt as to the relative order of these three markers, the orientation of this sequence with respect to the outside markers in genes 49 and e could be reversed. Detailed results were not provided by Tessman & Greenberg (1972) when they reported that the nrdC gene maps between genes 49 and rI.

The *Escherichia coli* deoxythymidine kinase can be allosterically regulated by both deoxythymidine triphosphate and deoxycytidine triphosphate, deoxythymidine triphosphate causing feedback inhibition while deoxycytidine triphosphate stimulates the activity (Okazaki & Kornberg, 1964b). This regulation permits a fine control of deoxythymidine kinase activity depending on the relative intracellular concentrations of the pyrimidine triphosphates. The T4-induced enzyme, on the other hand, is unaffected by deoxycytidine triphosphate and is inhibited by deoxythymidine triphosphate to a lesser extent. These differences might reflect the altered intracellular environment following phage infection. Since deoxycytidine triphosphate is destroyed by the T4-induced deoxycytidine triphosphatase (Warner & Barnes, 1966) there would appear to be no requirement for an enzyme.
regulation site for this compound. Moreover, since the rate of DNA synthesis increases after T4 infection the infected cells would require a larger supply of the immediate precursor, deoxythymidine triphosphate, which would necessitate the deoxythymidine kinase being less subject to feedback inhibition.

The induction of deoxythymidine kinase activity is a widespread occurrence in eukaryotic cells following infection by animal viruses. In some cases this induction represents the derepression of the host activity, e.g. polyoma virus (Littlefield & Basilico, 1966), while in others the induced enzyme, as with the T-even phages, is virus-specified, e.g. vaccinia virus (Dubbs & Kitt, 1964) and herpes simplex virus (Kit, Dubbs & Anken, 1967). The reasons for this induction are not clear but at least for herpes simplex virus some preliminary evidence suggests that the virus-induced deoxythymidine kinase is required for replication in resting cells (A. T. Jamieson, unpublished results). This would imply that under natural conditions of infection the induction of deoxythymidine kinase takes on the role of an essential function.

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