Isolation of a Specialized Transducing Bacteriophage Lambda Carrying the PolC* Locus of Escherichia coli

(H. attachment site/tonA/ΔpolC/DNA polymerase III)

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ABSTRACT  We have isolated a specialized transducing phage carrying the polC locus of E. coli K-12. A strain of E. coli lacking the lambda attachment site was infected with bacteriophage λ. Lysogens carrying λ at the tonA locus were isolated by selecting λ-immune, T5-resistant strains. Transducing phages of dapC, dapD and polC, which map within 0.2 min of tonA, were obtained in lysates prepared from two of the lysogens. The isolated phage, ΔpolC, is defective but can transduce four different polC temperature-sensitive mutants. After lysogenization with the transducing phage, DNA polymerase III activity is restored to normal levels in extracts of a polC strain lacking polymerase III activity. However, attempts to obtain increased levels of DNA polymerase III in extracts of induced lysogens carrying ΔpolC have been unsuccessful.

Three distinct DNA polymerases have been identified and purified from extracts of Escherichia coli (2). Furthermore, E. coli polA, polB, and polC mutants have been isolated which have defective DNA polymerases I, II, and III, respectively. Although the specific roles of the three polymerases in vivo have yet to be defined, DNA polymerase III is an essential enzyme in DNA replication. E. coli mutants having a thermostable mutation in the polC locus are unable to replicate DNA in vivo at the nonpermissive temperature and are also known to contain an altered polymerase III in vitro (3). Since a thermolabile enzyme is found in at least one of the polC mutants, the polC gene is the structural gene for polymerase III.

We report here the isolation of a specialized transducing phage carrying the polC locus. Bacteriophage lambda DNA normally integrates into the E. coli chromosome at a specific site (attBB'). Upon induction these lysogens produce, at a low frequency, transducing phages which carry only those bacterial genes that are adjacent to the prophage attachment site (4). Recently a method has been developed which allows insertion of lambda into other sites on the bacterial chromosome in mutants lacking the normal attBB' (5). However, the integration frequency of phage lambda into an attBB' deletion mutant chromosome is reduced 200-fold relative to that integrated into a wild-type E. coli chromosome. Analysis of the abnormal lysogens reveals several secondary attachment sites on the bacterial chromosome where insertion of lambda in or near specific genes has inactivated their function (5). This suggests the more general approach of specifying the site of abnormal lysogenization by simultaneously infecting attBB' mutants with lambda and selecting for the inactivation of a specific gene. The proximity of the tonA gene to the polC gene provided a selection for inserting phage λ near the polC gene and enabled us to isolate a specialized transducing phage carrying the polC locus.

MATERIALS AND METHODS

Bacterial and Phage Strains. E. coli strains used are all K-12 derivates and are described in Table 1. Phage P1ke was used for generalized transduction. Phage T5 wild type was employed for the selection of T5-resistant E. coli strains. Phage λcl604 synthesizes a thermolabile repressor inactive at 42°.

Media. L-Broth contained 1% Difco Bacto-Tryptone, 1.0% NaCl, 0.5% Bacto-Yeast Extract, 0.1% glucose, and 10 μg/ml of thymine. T-Broth contained 1% Difco Bacto-Tryptone, 0.5% NaCl, and 10 μg/ml of thymine. DL-α,ε-Diaminopimelic acid was present at 40 μg/ml where necessary. T-Broth agar plates and L-Broth agar plates contained 1.0% and 1.5% agar, respectively. T-soft agar contained 0.65% agar and L-soft agar contained 0.5% agar. L-Broth agar plates contained 2.5 mM CaCl2 for growth of phages P1kc and T5.

Chemicals. [3H]dTTP, dATP, dCTP, and dGTP were obtained from Schwarz BioResearch. Salmon sperm DNA and dl-α,ε-diaminopimelic acid were purchased from Sigma Chemical Co.

Table 1. Bacterial strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT982</td>
<td>Hfr KL16, dapD4, thi1</td>
<td>A. Taylor</td>
</tr>
<tr>
<td>AT997</td>
<td>Hfr KL16, dapC15, thi1</td>
<td>A. Taylor</td>
</tr>
<tr>
<td>HS353</td>
<td>ΔpolA/ΔpolC/ΔpolB</td>
<td>H. Shizuya</td>
</tr>
<tr>
<td>BT1026</td>
<td>polA1, polC3, endI, thi-</td>
<td>F. Bonhoeffer</td>
</tr>
<tr>
<td>BT1126</td>
<td>polA1, polC11, endI, thi-</td>
<td>F. Bonhoeffer</td>
</tr>
<tr>
<td>E486</td>
<td>polC3, thr-, leu-, thi-, met-</td>
<td>H. Hirota</td>
</tr>
<tr>
<td>JW108</td>
<td>polC3, thr-, leu-, thi-, met-</td>
<td>J. Wechler</td>
</tr>
<tr>
<td>HS453</td>
<td>polC3, thr-, dapD4, thi-, met-</td>
<td>H. Shizuya</td>
</tr>
</tbody>
</table>

Abbreviations: LFT, low frequency transductant; HFT, high frequency transductant; PFU, plaque-forming unit.

* In accordance with Taylor and Trotter (1) the gene symbol, polC, is used instead of dnaE to designate the gene for DNA polymerase III.

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**P1 Transduction Procedure.** Transductions were carried out as described by Lennox (6). Phage P1κ, grown on an appropriate donor, was added to cells \((5 \times 10^9/\text{ml})\) grown in L-Broth containing 2.5 mM CaCl₂ at a multiplicity of infection of 1.0. After 20 min the cells were collected by centrifugation, resuspended into a solution containing 10 mM MgSO₄, and plated onto appropriate plates.

**λ Transduction Procedure.** The recipient cells were grown to late log phase \((2 \times 10^9 \text{ cells per ml})\) in T-Broth, collected by centrifugation, resuspended in the same volume of 10 mM MgSO₄, and then incubated for 60 min at ambient temperature. After adjusting the concentration of cells to 5 \(\times 10^9/\text{ml}\), the cells were infected with transducing phages at various multiplicities along with helper phage \(λ^+\) at a multiplicity of five. The infected cells were incubated at ambient temperature for 15 min, washed by centrifugation, and plated onto appropriate plates.

**Integration of Lambda into the tonA Locus.** Log phase cultures \((40 \text{ ml})\) of *E. coli* HS353 in T-Broth were centrifuged, the cells were resuspended in 10 ml of 10 mM MgSO₄, and then starved for 30 min at 37° with shaking. The cells were infected with \(\lambda\)clAR at a multiplicity of four for 15 min at 20°. The infected cells were collected by centrifugation and resuspended in 80 ml of T-Broth. The culture was incubated 2 hr at 30° and centrifuged to remove phages released from the infected cells. The infected cells were resuspended in 175 ml of T-Broth and grown at 30° to allow time for the expression of the *tonA* phenotype. After 150 min the cells were collected by centrifugation to separate any remaining free phages and resuspended in 100 ml of T-Broth containing 1 mM CaCl₂. Phage T5 was added to the suspension at a multiplicity of infection of ten, and the cells were incubated for 60 min at 30°. After lysis the solution was centrifuged, and the pellet containing unlysed cells was resuspended in 4 ml of 0.85% NaCl; 0.25 ml was spread on T plates having 9 \(\times 10^9\) T5 phage on the surface, and the plates were incubated at 30° overnight. Each plate contained approximately 300 colonies.

**Test for Sensitivity of Strains to Phage T5.** The sensitivity of strains to phage T5 was tested by horizontally streaking a suspension of cells on an L-Broth agar plate previously streaked vertically with a stock of T5 phage \((1 \times 10^9 \text{ phage per ml})\). When cells were sensitive to T5, lysis occurred at the cross section.

**Test for λ Lysogens.** The presence of λ phage was tested by incubating colonies at 42° to detect the killing effect of \(\lambda\)clAR. A small culture of cells was then induced by incubation at 42° for 15 min followed by incubation at 37° for 2 hr. A few drops of CHCl₃ were added to the culture, and appropriate dilutions were plated with \(10^8\) *E. coli* C600 in 2 ml of T-soft agar on T-Broth agar plates. The plates were incubated at 37° to detect viable phage plaques.

**Preparation of LFT and HFT Lysates.** Low frequency transducing lysates (LFT) were prepared by heating the λ lysogens at 42° for 15 min and then incubating at 37° until lysis. High frequency transducing lysates (HFT) were obtained by inducing heterogenotes both with ultraviolet light and with heat simultaneously.

**Purification of DNA Polymerase III.** Cells were grown in 500 ml L-Broth at 30° until late log phase and then harvested by centrifugation at 5000 \(\times g\) for 30 min at 0°. The cells were stored at \(-30°\). Frozen cells \((1 \text{ g})\) were resuspended in 5 ml of 20 mM KPO₄ buffer \((pH 6.8)-10\% \text{ (w/v)}\) glycerol-10 mM mercaptoethanol and then passed through a French Press at 6000 lb/inch². The crude extract was centrifuged for 30 min at 27,000 \(\times g\) to remove cell debris, and the supernatant fluid was diluted with the same buffer to adjust A₂₆₀ to 200. Streptomycin sulfate \((30\% \text{ (w/v)})\) was added slowly to a final concentration of 3% \(\text{(w/v)}\) and stirred for 30 min. The suspension was centrifuged for 30 min at 27,000 \(\times g\) and the pellet was discarded. Solid ammonium sulfate \((0.243 \text{ g/ml})\) was added slowly and stirred for 30 min before centrifugation at 27,000 \(\times g\) for 30 min. The resulting pellet was resuspended in a small volume \((\text{approximately 2.5 ml})\) of KPO₄ buffer \((pH 6.5)-25\% \text{ (w/v)}\) glycerol-10 mM mercaptoethanol and diluted until the conductivity indicated less than 30 mM ammonium sulfate. The protein was applied to a phosphocellulose column \((1 \text{ cm}^2 \times 5 \text{ cm})\) previously equilibrated with the above buffer. After the column was washed with 15 ml of the same buffer, a linear gradient of KPO₄ buffer \((pH 6.5)\) from 0.02 to 0.40 M containing 25% \(\text{(w/v)}\) glycerol and 10 mM mercaptoethanol was pumped through the column, and 1 ml fractions were collected.

**Assay for DNA Polymerase III.** Reaction mixtures \((300 \text{ μl})\) contained: 20 mM Tris- HCl \((pH 7.2)\), 13.3 mM MgCl₂, 2 mM dithiothreitol, 33 μM each dATP, dCTP, dGTP, and [γH]dTTP \((100 \text{ cpm/μmole})\), 8.3 mg/ml of salmon sperm DNA, and 0.001–0.01 unit of enzyme. After incubation for 30 min at 30°, the reaction was stopped by the addition of 3 ml 1 N cold trichloroacetic acid; the DNA was collected on a filter, and the radioactivity was determined as previously described (7). A unit of DNA polymerase III activity is defined as the amount catalyzing the incorporation of 10 nmoles total nucleotides into acid-insoluble material during the time of incubation.

**RESULTS**

**Insertion of λclAR Near the polC Locus.** Insertion of a phage chromosome, such as that of phage λ, into the *E. coli* chromosome near the *polC* gene should allow the isolation of λ phages carrying a portion of the bacterial chromosome including the *polC* locus. The *polC* mutants isolated by Wechsler and Gross (8) are 54–99% cotransducible with *tonA* which is located at 2.5 min on the *E. coli* genetic map. Selection for T5 resistance and thus prophage insertion into *tonA* should allow the isolation of a strain carrying phage λ near *polC*.

Of approximately 1000 T5-resistant colonies obtained from a λclAR infection of *E. coli* HS353 lacking the lambda attachment site, five were found to be lambda lysogens, the remainder being spontaneous mutants. Phage P1 transduction experiments showed that two of these, *E. coli* AR252 and AR-508, contained lambda inserted into or near the *tonA* locus. The frequency with which phage P1, grown on each of these strains, cotransduces *dapD*, a marker located 0.2 min to the right of *tonA*, and phage lambda is shown in Table 2. In the case of AR508, we could not separate lambda immunity from T5 resistance, suggesting that λ inserted directly into *tonA*. In the other lysogen, AR252, λ immunity and T5 resistance could be separated by P1 transduction. The mapping data presented in Table 2 indicate that in this strain, lambda is closer to *dapD* than it is in AR508. Thus, lambda is probably inserted just to the right of *tonA*. In this case it is not clear
Table 2. Cotransduction of prophage \( \lambda \) and \( \text{dapD}^+ \)

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>( \text{dapD}^+ ) transductants</th>
<th>( \text{dapD}^+\lambda^- )</th>
<th>( \text{dapD}^+\lambda^+ )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta R252 )</td>
<td>AT982</td>
<td>( 2.2 \times 10^{-7} )</td>
<td>17/24</td>
<td>7/24</td>
</tr>
<tr>
<td>( \Delta R508 )</td>
<td>AT982</td>
<td>( 3.5 \times 10^{-4} )</td>
<td>134/157</td>
<td>23/157</td>
</tr>
</tbody>
</table>

The recipients were treated with lysates of P1kc grown on donors at a multiplicity of 0.1 and plated on T-Broth plates at 30° to select for \( \text{dapD}^+ \). The presence of \( \lambda \) prophage was then scored by replica plating at 42°.

whether the inactivation of \( \text{tonA} \) is related to the insertion of lambda or to an independent event. On the basis of the two lysogens isolated, the frequency of insertion of lambda into \( \text{tonA} \) is less than \( 10^{-4} \). A partial genetic map of \( \text{E. coli} \) showing the region where lambda is inserted is presented in Fig. 1.

Isolation of a \( \lambda\text{dpolC} \) Transducing Phage. Because the \( \text{dapD} \), \( \text{dapC} \), and \( \text{polC} \) genes are very close to the prophage lambda, it should be possible to obtain lambda phage bearing a substitution of bacterial genes for a portion of the prophage chromosome by abnormal excision in a manner analogous to the preparation of \( \lambda\text{bio} \) and \( \lambda\text{gal} \) (4). Lysates obtained by heat induction of \( \text{E. coli} \) \( \Delta R252 \) and \( \Delta R508 \) were tested for their ability to transduce \( \text{dapD} \), \( \text{dapC} \), and \( \text{polC} \) strains with the aid of helper phage (\( \lambda^+ \)). Lysates obtained from \( \Delta R252 \) transduced \( \text{dapD} \) at a frequency of \( 10^{-7}/\text{PFU} \) (plaque-forming unit), \( \text{polC} \) at \( 10^{-4}/\text{PFU} \), and \( \text{dapC} \) at \( 10^{-6}/\text{PFU} \) with helper phage included at a multiplicity of infection of five. The relatively high transducing frequencies and the low plaque-forming titer (10/ml) of the LFT lysates probably reflect the abnormal excision of the prophage from the \( \text{tonA} \) locus on the host chromosome.

Some Genetic Properties of the Transducing Phage. Heat and ultraviolet induction of lysogens obtained from the infection of \( \text{polC}^+ \) strains with LFT lysates plus helper phage yielded HFT lysates in more than 70% of the colonies checked. In five HFT lysates examined the transducing titer was 100 times lower than the active phage titer, as shown for one such lysate in Table 3. Furthermore, superinfection with \( \lambda\text{i21} \) of lysogens producing HFT lysates could cure the lysogens of the \( \lambda\text{dpolC} \) phage at a frequency of approximately 1 in 10. These results imply that \( \lambda\text{dpolC} \) is integrated at the lambda attachment site in HFT producing lysogens.

Attempts to find transducing phage capable of forming plaques proved unsuccessful. When phage from over 200 plaques produced by HFT lysates were tested for their ability to transduce \( \text{polC} \), none were capable of transduction with or without helper phage. In transduction experiments where a low multiplicity of phage from a HFT lysate was used, a small number of transductants appear. These proved to be single lysogens of transducing phage which were capable of cell lysis upon induction, but incapable of producing phage able to form plaques. We conclude that the transducing particle is a defective phage.

\( \lambda\text{dpolC} \) probably carries the entire \( \text{polC} \) gene. All of the temperature-sensitive \( \text{polC} \) mutants tested could be transduced by the \( \lambda\text{dpolC} \) phage. These strains were derived from independent genetic backgrounds, and each \( \text{polC} \) mutation gives rise to a distinct altered DNA polymerase III activity in extracts (3). Purified enzyme from \( \text{E. coli} \) BT1026 is active at 30°, but inactive at 45°, whereas no activity is detected at any temperature in extracts of \( \text{E. coli} \) E486; DNA polymerase III purified from \( \text{E. coli} \) JW108 is no more thermostable than that purified from wild-type W3110. The lower transducing frequencies of \( \text{polC} \) in BT1026 and BT1126, as compared to E486 and JW108, could be due to the \( \text{polA} \) mutation. More \( \text{polA}^- \) than \( \text{polA}^+ \) cells are killed by lambda infection, and consequently the frequency of lysogenization by normal phage is less in \( \text{polA}^- \) than in \( \text{polA}^+ \) (unpublished results).

Restoration of DNA Polymerase III Activity by \( \lambda\text{dpolC} \). When the mutant \( \text{E. coli} \) E486, bearing a \( \text{polC} \) mutation, is transduced by \( \lambda\text{dpolC} \), the level of polymerase III activity is

Table 3. Transduction of \( \text{E. coli} \) \( \text{polC} \) mutants by \( \lambda\text{dpolC} \)

<table>
<thead>
<tr>
<th>Recipient strain</th>
<th>( \text{polC} ) mutation</th>
<th>Transductants/\text{PFU}</th>
</tr>
</thead>
<tbody>
<tr>
<td>E486</td>
<td>( \text{polC}^+ )</td>
<td>( 1.0 \times 10^{-1} )</td>
</tr>
<tr>
<td>JW108</td>
<td>( \text{polC}^+ )</td>
<td>( 1.0 \times 10^{-1} )</td>
</tr>
<tr>
<td>BT1026</td>
<td>( \text{polC}^+ )</td>
<td>( 1.0 \times 10^{-1} )</td>
</tr>
<tr>
<td>BT1126</td>
<td>( \text{polC}^+ )</td>
<td>( 1.0 \times 10^{-1} )</td>
</tr>
</tbody>
</table>

Transduction was carried out as described in Materials and Methods. The recipients were infected with \( \lambda\text{dpolC} \) and \( \lambda^+ \) and plated on T-broth agar plates incubated at 42°.

Fig. 1. Abnormal insertion of \( \lambda \) near \( \text{polC} \) in strains \( \Delta R252 \) and \( \Delta R508 \). The shaded regions on the maps indicate the extent of the deletion which includes the normal \( \lambda \) attachment site.
restored to that found in extracts of polC+ cells. The purification scheme described under \textit{Materials and Methods} has been designed to remove DNA polymerases I and II activities present in \textit{E. coli} E486. The peak of activity, which elutes between 0.12 M and 0.18 M KPO4 on the phosphocellulose column shown in Fig. 2, is judged to be DNA polymerase III by its sensitivity to N-ethylmaleimide and high ionic strength, its resistance to antibody made against DNA polymerase I, and its inability to use poly[d(A-T)] as a template for polymerization (C. C. Richardson and D. M. Livingston, unpublished results). As shown in Fig. 2, no polymerase III activity can be found during chromatography of a partially purified extract of \textit{E. coli} E486 on phosphocellulose. However, after transduction normal amounts of polymerase III are found on the phosphocellulose chromatogram at the position expected for polymerase III purified from wild-type \textit{E. coli} (Fig. 2). Furthermore, after the lysogen has been cured of the transducing phage, no polymerase III can be detected. Although the lysogens contain normal levels of polymerase III, induced cells do not show the same increase in production of polymerase III as they do of phage proteins. All of our attempts to obtain increased levels of DNA polymerase III with these lysogens have been unsuccessful.

\textbf{DISCUSSION}

The integration of \(\lambda\) into or near the tonA gene may differ from the process of secondary site integration described by Shimada \textit{et al.} (5). They found that the int function of lambda is necessary for secondary site integration as it is for normal integration. This suggests that there are several sites on the bacterial chromosome besides the attachment site (att\(\lambda\)) where \(\lambda\) can preferentially integrate. The integration of lambda into or near tonA is extremely inefficient compared to secondary site integration, suggesting that the integration sites in or near tonA are not of the same class as those described by Shimada \textit{et al.} (5). Thus, the \textit{int} function of lambda may not be necessary for integration, but integration might proceed by another pathway, perhaps as seen during illegitimate recombination (9).

Both strains \(\Delta R508\) and \(\Delta R252\) yield transducing phage of the \textit{polC} gene. These phage occur at a frequency of \(10^{-4}\) from \(\Delta R252\) lysates, implying abnormal excision of the prophage. The ability of these phages to transduce four different \textit{polC} temperature-sensitive mutants and to restore wild-type polymerase III activity strongly suggests that the entire \textit{polC} locus can be carried by phage derived from insertion sites in or to the right of tonA.

Although bacterial genes carried by transducing phage often show a gene dosage effect, the induction of \(\lambda d p o l C\) lysogens does not increase the activity of DNA polymerase III relative to the uninduced conditions. One explanation is that the \textit{polC} transducing phage cannot be replicated after induction because it lacks the phage replicative origin, and thus only a single transducing phage per bacterium is ever produced. A second possibility is that the direction of transcription of the \textit{polC} gene is blocked by the transcription of lambda operons in the opposite direction after induction. Another possibility is that the production of DNA polymerase III is strictly regulated, and excessive amounts of polymerase III may not be produced. In fact, it has been shown that a specialized transducing phage carrying the \(\beta\) subunit of RNA polymerase produces only a 2-fold increase in the amount of \(\beta\) subunit synthesis, and no gene dosage effect is observed in the uninduced lysogens even if the cell is diploid (10, 11).

The isolation of \textit{polC} transducing phage should provide a useful tool for biochemical and biological studies of DNA polymerase III biosynthesis. The method described here extends previous techniques (5, 12, 13) for isolating specific transducing phage, and may be useful to others seeking to place genes on transducing phages.

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