Resolution of the DNA Strands of the Specialized Transducing Bacteriophage λh80C,857dargF

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The DNA strands of lambdoid phages with deletions or substitutions of the guanine plus cytosine-rich region in the left arm are not resolvable by complexing with poly UG followed by centrifugation in CsCl. This work describes a completely general procedure for the strand resolution of these phages by hybridization with fragments of separated strands of the parent phage. In particular, resolution of the DNA strands of the specialized transducing phage λh80C,857dargF is described, and evidence is presented which indicates that argF is transcribed from the r strand.

Genetic information is, with few exceptions, encoded by double-stranded DNA with one strand complementary to the other as first proposed by Watson and Crick (21). The specific information for any given gene is defined by one of the two strands. All sense information may be encoded by one strand as in the case of bacteriophage T3 and T7 (17, 18); however, in general some genes are transcribed from one strand and other genes are read in the opposite direction from the other strand. This has been clearly demonstrated for the coliphages λ and T4 (5, 19), and in some cases particular regions are transcribed from both DNA strands (1). The direction of transcription of a number of genes in *Escherichia coli* has been determined (20), and of particular interest is the divergent transcription of the argECBH genes of the arginine regulon. It has been shown that argE is transcribed anticlockwise from a control region between argE and argC and that the argCBH genes are transcribed in the opposite direction from a closely linked control region (7, 11, 16). The determination of the orientation of transcription of various genes and the detailed study of their regulation in vitro have been made possible by the availability of techniques for the resolution of the DNA strands of various bacteriophages.

The method of choice for resolving the DNA strands of λ, φ80, and T7 is to complex the DNA with a ribopolymer, poly UG, followed by centrifugation to equilibrium in a CsCl gradient as described by Hradecna and Szybalski (10). In the case of λ this procedure takes advantage of a guanine plus cytosine-rich region on the left arm; however, λ mutants with deletions in this region and specialized transducing bacteriophages (λh80C,857dara [9], λh80C,857dhisgnd [22], λh80C,857dargF [17, James and L. Gorini, Fed. Proc. 31:3710, 1972]) which have this region replaced by bacterial genes have DNA which is not amenable to strand separation by established procedures.

We require resolved strands of λh80C,r857dargF for studying the regulation of gene expression in the arginine biosynthetic pathway. A facile technique for resolving the DNA strands of any φ80 or λ phage is described, and in particular we report that the r strand of λh80C,857dargF carries the sense information for argF.

MATERIALS AND METHODS

**Materials.** Ready-Solv IV and VI scintillation fluid and scintillation vials were obtained from Beckman Instruments, Atlanta, Ga. All radioisotopes were purchased from New England Nuclear Corp., Boston, Mass. Electrophoretically purified DNase and RNase were obtained from Worthington Biochemicals Corp., Freehold, N.J. Selectron B6 filters were obtained from Arthur Thomas. Media was purchased from Difco Laboratories, Detroit, Mich. Cesium chloride was obtained from Columbia Organic Chemicals, Columbia, S.C. Poly UG was purchased for Biopolymers, Inc. and had a U-G ratio of 1.9:1.

**Media.** Bacteria used for preparing phage stocks were grown in L liquid medium (13) or L agar plates except for the preparation of 32P-labeled phage when bacteria were grown in S medium (Table 1). Bacteriophages were titered on H plates in H top agar (9). F top agar was used for plating cells on minimal medium (14). Selection plates contained medium A (6) and supplemental growth factors as required, 2% agar, and 0.5% glucose as carbon source. Supplements were used at the concentrations previously described (8).

**Bacteria and bacteriophage.** Bacterial strains and bacteriophage used in this work are listed in Table 2.

**Propagation of bacteriophage.** λCc2 was propa-
gated by the plate procedure using the sensitive bacterial strain CA8000; \( \lambda h80C_{857} \) and \( \lambda C_{857}S7 \) were prepared from appropriate lysogenic strains as described by E. James and L. Gorini (manuscript in preparation).

Bacteriophage labeled with \( ^{32}P \) were prepared from lysogens grown in S1 (Table 1) medium with 2 mM \( KH_2PO_4 \). Cells were grown to an optical density at 575 nm (OD_{575}) of 0.40, at which time they were sedimented by centrifugation at 8,000 rpm for 4 min; the resulting pellet was rapidly resuspended in S2 (Table 1) medium, grown at 42 C for 15 min, transferred to 37 C, and grown until confluent lysis had occurred. Phage DNA with a specific activity of 1.0 \( \times 10^{10} \) to 7.0 \( \times 10^{10} \) counts per min/\( \mu g \) was prepared as described except the Casamino Acids solution used in both S1 and S2 media was treated with 1.90 ml of 1.0 M calcium chloride per 25 ml of 20% Casamino Acids, and precipitated calcium phosphate was removed by centrifugation at 6,000 \( \times g \) for 10 min.

**Purification of bacteriophage.** Phage lysates were clarified by centrifugation at 8,000 rpm for 15 min, and phage was sedimented for 12 to 16 h in the presence of 10% polyethylene glycol 6000 (Arthur Thomas) and 0.5 M NaCl as described by Yamamoto et al. (24). The sediment was collected by centrifugation at 5,000 rpm for 5 min and the pellet was carefully resuspended in \( \frac{1}{50} \) of the original lysate volume of T1 buffer (6 \( \times 10^{-14} \) M MgSO_4, 5 \( \times 10^{-4} \) M CaCl_2, 6 \( \times 10^{-3} \) M Tris-hydrochloride, pH 7.3, 0.1% [wt/vol] gelatin). The concentrated phage was sedimented for 2 h at 30,000 rpm in a cushion comprising 2 ml of 1.4-density CsCl above 2 ml of 1.6-density CsCl using a 50.2 Ti rotor in the Beckman ultracentrifuge. Phage were further purified by repeated centrifugation in a cesium chloride density gradient using 1.4, 1.5, 1.6 block gradients for a period of 4 to 6 h, 1.4, 1.6 block gradients for 12 to 16 h, or running to equilibrium in 1.5-density CsCl using either the 50.2 Ti, 50 Ti, or 75 Ti angle rotors. After centrifugation, gradients were harvested by upward displacement by fluorescent FC 40 (Minnesota Mining and Manufacturing Co., St. Paul, Minn.) with an ISCO gradient fractionator coupled to a Chromatronics dual-wavelength absorbance monitor with an 0.5-mm path length flow cell.

**Resolution of phage DNA strands by complexing with poly UG.** Strand resolution of phage DNA by complexing with poly UG was performed as described by Hrada and Szybalski (10) and Szybalski (personal communication). Cesium chloride was added to high purity water to give a saturated solution at 60 C, and the warm solution was rapidly filtered through a 0.45-\( \mu \)m membrane filter (Millipore). Saturated CsCl solutions had an OD_{260} of less than 0.010. Polyallomer centrifuge tubes (1/3 by 3 inch; ca. 1.7 by 7.6 cm) were placed in 1 liter of 0.1 M EDTA, pH 10.0, and heated to 100 C for 15 min; the tubes were washed with glass distilled water and stored at room temperature. A quantity of phage containing 350 to 450 \( \mu g \) of DNA, freshly purified in a CsCl gradient, with an OD_{260} greater than 60, was placed into a dialysis bag, both ends were closed with hemostats, and the phage was dialyzed for 2 h against 1 liter of phage dialysis buffer at 4 C. The phage were transferred to a test tube (16 by 125 mm), and the volume was adjusted to 1.83 ml with dialysis buffer; 0.50 ml of an aqueous solution of poly UG (1 mg/ml) and 0.015 ml of 0.1% Sarkosyl NL97 were added, and the mixture was heated for 2.5 min at 95 C in a hot water bath with gentle swirling for the first 30 s. The mixture was rapidly cooled to 5 C followed by the addition of 0.45 ml of 0.5 M Tris-hydrochloride (pH 7.6) and 10.9 ml of saturated CsCl. The density was adjusted to 1.725 (refractive index 1.4022), and the mixture was centrifuged in a 75 Ti or 50 Ti rotor at 35,000 rpm for 67 to 72 h at 17 C. At the termination of the run each preparation was fractionated as described for the isolation of phage.

The separated strands were dialyzed overnight against 140 mM phosphate buffer (pH 7.6), adjusted to 0.3 N KOH, and incubated for 12 h at 37 C to remove poly UG. The reaction mixture was neutralized with 3 N HCl, annealed at 68 C for 3 h and passed through a 5-ml hydroxyapatite column at 60 C to remove double-stranded DNA. Single-stranded DNA was dialyzed against 1 mM EDTA (pH 8.0) and stored at 4 C over a drop of chloroform.

**Isolation of DNA from the phage \( \lambda h80C_{857} \).** Bacteriophage was purified immediately prior to isolation of DNA by centrifugation to equilibrium in 1.5-density cesium chloride, and purified phage was adjusted with 1.5-density cesium

### Table 1. Composition of S1 medium

<table>
<thead>
<tr>
<th>Contents</th>
<th>Conc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris-hydrochloride (pH 7.4)</td>
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<tr>
<td>NaCl</td>
<td>7 mM</td>
</tr>
<tr>
<td>NH_4Cl</td>
<td>10 mM</td>
</tr>
<tr>
<td>KH_2PO_4</td>
<td>3 mM</td>
</tr>
<tr>
<td>MgSO_4</td>
<td>2 mM</td>
</tr>
<tr>
<td>CaCl_2</td>
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<tr>
<td>Vitamin B_1</td>
<td>4 mg/liter</td>
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<tr>
<td>20% Glucose</td>
<td>20 ml/liter</td>
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</tbody>
</table>

* S1 and S2 medium also contained all amino acids and nucleic acid bases at the concentrations described (8). S2 medium has the same composition except for the omission of phosphate.

### Table 2. List of strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA8000 Hfr thi</td>
<td>J. Beckwith</td>
</tr>
<tr>
<td>EJ 81 thi thr leu argF argI gal lac</td>
<td>Our collection</td>
</tr>
<tr>
<td>EJ 83.1 thi thr leu argF argI gal lac str+ (( \lambda h80C_{857} ))</td>
<td>Our collection</td>
</tr>
<tr>
<td>EJ 107 thi argI argR_{18} spec</td>
<td>Our collection</td>
</tr>
<tr>
<td>( \lambda C_{857} )C</td>
<td>W. Szybalski</td>
</tr>
<tr>
<td>( \lambda h80C_{857} )S7</td>
<td>Our collection</td>
</tr>
<tr>
<td>( \lambda h80C_{857} )dargF</td>
<td>Our collection</td>
</tr>
<tr>
<td>( \lambda C_{857}S7 )</td>
<td>N. Kelker</td>
</tr>
</tbody>
</table>
chloride to yield a concentration corresponding to an OD$_{560}$ of 13. The phage solution was dialyzed against 1 × SSC (0.15 M NaCl plus 0.015 M sodium citrate, pH 7.4) for 3 h without stirring, and the phage was extracted three times with an equal volume of redistilled phenol saturated with 1 × SSC. For the first extraction 15 μl of a 25% solution of sodium dodecyl sulfate was added per ml. The third phenol extraction was followed by extraction with chloroform-isoamyl alcohol (24:1, vol/vol); the aqueous phase was adjusted to 25 μg/ml in Pronase and incubated for 2 h at 37 C. Two additional phenol extractions were performed followed by a final chloroform-isoamyl alcohol extraction. The aqueous layer was dialyzed exhaustively against 10 mM EDTA, pH 8.0, and stored at 4 C over a drop of chloroform.

**Preparation of 4 to 5S single-stranded DNA fragments.** A quantity of single-stranded DNA, usually 800 μg, in 13 ml of 1 mM EDTA was sonically treated with a Bronson Heat Systems sonifier at a power output of 85 W using four pulses of 30-s duration with 5-min cooling at 0 C between each pulse. The fragments were concentrated to a volume of 3 ml by centrifugal lypohilization in a siliconex corex tube, and 0.5-ml aliquots were layered on a 5 to 30% sucrose gradient (0.5 M NaCl-1 mM EDTA) with a 0.5-ml cushion of 50% sucrose and centrifuged in an SW50.1 rotor at 40,000 rpm to an a value of 55,000. The gradients were fractionated from the bottom; the upper 2.0 ml contained 4 to 5S fragments which were pooled, concentrated by centrifugal lypohilization to a volume of 5 ml, and desalted by gel filtration on a Sephadex G15 column equilibrated with 1 mM EDTA. DNA larger than 4 to 5S, recovered from the bottom of the gradient, was used in subsequent solification experiments.

**Hydroxyapatite chromatography.** Denatured and native DNA were separated by differential adsorption to and elution from hydroxyapatite (BioRad, Richmond, Calif., Bio Gel control no. 11704) according to Kohne and Britten (12). Columns of hydroxyapatite were poured in disposable plastic syringe barrels in a 60 C water bath (J. F. Sambrook, personal communication). Mixtures of native and renatured DNA were applied in 140 mM phosphate buffer (pH 7.0). Single-stranded DNA passed through the column, which was washed with eight column volumes of 140 mM phosphate buffer. Duplex DNA was eluted with six column volumes of 400 mM phosphate buffer.

**Determination of radioactivity.** Bovine serum albumin was added as carrier for samples which were precipitated with 10% trichloroacetic acid. The precipitates were collected on glass fiber filters (Whatman), dried, and counted in Ready-Solv IV scintillation fluid. Samples containing 3P were counted without fluor by Cerenkov radiation in a Beckman LS 290 scintillation spectrometer.

**Hybridization conditions.** All hybridizations were performed at 68 C in 140 mM phosphate buffer (pH 7.6)-0.4% sodium dodecyl sulfate. Native DNA was denatured at low salt concentration by heating to 97 C for 4 min, quick cooled, adjusted to 140 mM phosphate, and passed through a hydroxyapatite column to remove any tangled DNA. In experiments to determine the kinetics of reassociation, samples were withdrawn from the annealing mixture at appropriate times, diluted 10-fold into 140 mM phosphate buffer, and stored at 4 C until the proportion of single- and double-stranded DNA was determined by chromatography on hydroxyapatite.

**Preparation of argF mRNA.** Strain EJ107 was grown at 37 C in S1 medium without uridine to a concentration of approximately 5 × 10⁴ cells/ml, at which time the culture was pulsed for 50 s by the addition of 4 μCi of [3H]Juridine per ml. At the end of the pulse period a freshly prepared solution of sodium azide was added to 0.02 M final concentration, and the culture was immediately poured on to the same volume of frozen crushed buffer containing 20 mM Tris-hydrochloride (pH 7.3)-5 mM MgCl₂. The cells were allowed to thaw, concentrated by centrifugation for 10 min at 8,000 rpm, and resuspended in a 0.1 volume of 0.1 × SSC (pH 7.0). The cells were lysed by a single passage through a French press at 8,000 lb/in². Bentonite was immediately added to the lysate to a final concentration of 0.01 mg/ml, the mixture was stirred gently at 4 C for 5 to 10 min, and bentonite was removed by centrifugation for 10 min at 13,000 rpm at 4 C.

DNase (RNase free) was added to the supernatant liquid at a concentration of 20 μg/ml, and the mixture was incubated at room temperature for 15 min. The solution of RNA was then extracted three times with phenol saturated with 0.1 × SSC. The first extraction was performed in the presence of 0.4% SDS. After each extraction the mixture was centrifuged at 6,000 rpm for 5 min to facilitate phase separation. After the last phenol extraction, residual phenol was removed from the aqueous phase by extraction with chloroform-isoamyl alcohol (24:1, vol/vol). The aqueous phase was heated in a boiling water bath for 4 min and quick chilled, and 20 × SSC was added to give an overall concentration of 2 × SSC. The cold solution was passed through a double layer of nitrocellulose filters. To the filtered solution a 0.1 volume of 20% sodium acetate (pH 5.2) and 2 volumes of ethanol (at −20 C) were added, and precipitation was allowed to occur overnight at −20 C.

The RNA precipitate was collected by centrifugation and resuspended in 0.1 × SSC and passed through a Sephadex G-50 (fine) column (0.9 by 30 cm, equilibrated with 0.1 × SSC). RNA eluted in the void volume and was concentrated by ethanol precipitation. RNA was resuspended in 2 × SSC, saturated with phenol, and stored at 4 C.

**RESULTS**

The resolution of the DNA strands of bacteriophage λ and φ80 is readily affected by complexing with poly UG followed by centrifugation to equilibrium in a 1.725-density cesium chloride gradient as shown in Fig. 1a and b for λCYP578 and λCβ2, respectively. Separations such as those shown in Fig. 1a and b are efficacious due to the specific complexing of
poly UG with the guanine plus cytosine-rich region of the left arm of the bacteriophage. In those cases in which this guanine plus cytosine-rich region is removed by a deletion or substitution, as in the case of $\phi 80dargF$ and $\lambda h80C,857dargF$, the guanine plus cytosine-rich region is no longer available to complex poly UG. The DNA isolated from such phages is not amenable to strand separation using poly UG (Fig. 1c). The work of Westphal (22) and of Sambrook et al. (16) with simian virus 40 and the following considerations suggest an alternate procedure for resolving the DNA strands of $\lambda h80C,857dargF$.

(i) DNA isolated from $\lambda h80C,857$ and $\phi 80$ may be readily resolved using poly UG.

(ii) Single-stranded $\lambda$ fragments should compete for the $\lambda h80C,dargF$, strand in a reassociation experiment with denatured $\lambda h80C,857dargF$.

(iii) The rate of reassociation of high concentrations of $\lambda$ fragments with the complementary strand of $\lambda h80C,857dargF$ should be faster than that of the corresponding strand of $\lambda h80C,857dargF$, in direct proportion to the increased concentration of fragments relative to $\lambda h80C,857dargF$.

(iv) The unit length-fragment DNA hybrid duplex may be separated from single-stranded DNA by chromatography on hydroxyapatite.

(v) Single-stranded fragments of 4 to 5S can be readily separated from unit length $\lambda h80C,857dargF$ DNA by centrifugation in a sucrose density gradient.

If unit length-denatured $\lambda h80C,857dargF$ DNA was hybridized to either $\lambda h80C,857 r$ or $l$ strand fragments of 4 to 5S, only one of the DNA strands of the specialized phage should hybridize to the 4 to 5S-resolved DNA. By performing the reaction with a large excess of 4 to 5S fragments, the rate of hybridization of fragments to their complement should be considerably faster than that of the homologous $\lambda h80C,857dargF r$ or $l$ strand; excess single-stranded fragments together with the same strand of $\lambda h80C,857dargF$ should not be retained when chromatographed on hydroxypatite at 140 mM phosphate, whereas unit length-fragment hybrid DNA would be retained and eluted at 400 mM phosphate.

Data are presented in Fig. 2 which summarize the results of a number of reassociation experiments in which $^3$H-labeled unit length $\lambda h80C,dargF$ DNA was denatured and permitted to reassociate in the presence of various concentrations of 4 to 5S fragmented r strand DNA isolated from $\lambda Cb2$. The fraction of radioactive single-stranded DNA present after hybridization had been permitted to proceed for varying times was ascertained by chromatography on hydroxyapatite. Increasing concentrations of $\lambda Cb2$ fragments lead to a decrease in the percentage of labeled duplex DNA, but even with a 30-fold excess of fragments less than 50% radioactive-labeled single-stranded DNA was present; however, the use of $\lambda h80C,857$ fragments or a mixture of fragments from $\lambda Cb2$ and

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Fig. 1. Comparison of efficacy of complexing with poly UG followed by centrifugation to equilibrium in 1.710-density cesium choride for the resolution of the DNA strands of various bacteriophage. (a) $\lambda Cb2$; (b) $\lambda h80C,857$; (c) $\lambda h80C,857dargF$.
and the solution was rapidly cooled to 0 C and applied to a 3-ml hydroxyapatite column maintained at 60 C, with occasional stirring of the hydroxyapatite column. The fraction passing through the column contained more than 90% of the single-stranded DNA; the remaining single-stranded DNA eluted with 10 ml of 140 

**Fig. 2.** Relationship between 4H-labeled single-stranded \( \lambda h80C_{1857}dargF \) present and concentration of added single-stranded \( \lambda Cb2 \) fragments. \( \lambda h80C_{1857}dargF \) DNA (1.0 \( \mu g \)) was denatured in 0.5 ml of 1 mM EDTA by heating at 97 C for 5 min and adjusted to a total volume of 2 ml in 140 mM phosphate buffer containing various concentrations of 4 to 5S \( \lambda Cb2 \) single-stranded DNA. The mixture was incubated at 68 C for 60 h, at which time 1 ml was removed, diluted with 9 ml of 140 mM phosphate buffer-0.4% sodium dodecyl sulfate, and stored at 4 C until assayed for single-stranded 4H-labeled DNA by chromatography on hydroxyapatite.

\( \phi 80 \) led to the rapid reassociation of 50% of the labeled DNA to the duplex form (Fig. 3 and 4) with 50% of the radioactive-labeled single-stranded DNA present. These data clearly indicate that annealing denatured DNA, isolated from a specialized transducing bacteriophage, with a 30-fold excess of single-stranded fragments from an appropriate phage, should permit separation of the strands of the specialized phage.

**Preparation of separated strands of \( \lambda h80C_{1857}dargF \).** The strands of \( \lambda h80C_{1857}dargF \) are separated routinely by using the following protocol: 5 \( \mu g \) of \( \lambda h80C_{1857}dargF \) DNA was diluted to 4.0 ml with sterile water, heated at 97 C for 5 min, and immediately cooled to 0 C. Purified 4 to 5S fragmented DNA (150 \( \mu g \)) isolated from \( \lambda h80C_{1857} \) strand DNA was added, and the mixture was adjusted to 140 mM phosphate (pH 7.6)-0.4% sodium dodecyl sulfate in a total volume of 10 ml. The mixture was incubated at 68 C for 1.5 h, and the solution was rapidly cooled to 0 C and applied to a 3-ml hydroxyapatite column maintained at 60 C, with occasional stirring of the hydroxyapatite column. The fraction passing through the column contained more than 90% of the single-stranded DNA; the remaining single-stranded DNA eluted with 10 ml of 140 

**Fig. 3.** Rate of conversion of single-stranded \( \lambda h80C_{1857}dargF \) DNA into duplex DNA in the presence of a 30-fold excess of single-stranded \( \lambda h80C_{1857} \) to 5S fragments. \( \lambda h80C_{1857}dargF \) DNA (2.0 \( \mu g \)) was denatured in 1 ml of 1 mM EDTA by heating at 97 C for 5 min and adjusted to a total volume of 4 ml in 140 mM phosphate buffer containing a 30-fold excess of 4 to 5S \( \lambda h80C_{1857} \) strand DNA. The mixture was incubated at 68 C. Samples (0.5 ml) were removed at intervals, diluted with 3.5 ml of 140 mM phosphate buffer-0.4% sodium dodecyl sulfate, and stored at 4 C until assayed for single-stranded 4H-labeled DNA by chromatography on hydroxyapatite.

**Fig. 4.** Rate of conversion of single-stranded \( \lambda h80C_{1857}dargF \) DNA into duplex DNA in the presence of a 20-fold excess of single-stranded \( \lambda h80C_{1857} \) to 5S fragments and a 10-fold excess of \( \lambda Cb2 \). Experimental conditions as described in the legend to Fig. 3.
mM phosphate buffer. Double-stranded DNA was eluted from the hydroxypatite column with 6 ml of 400 mM phosphate buffer (pH 7.6) with 0.4% sodium dodecyl sulfate; an additional 8 ml of the same buffer removed more than 95% of the double-stranded DNA.

Radioactive DNA was equally divided between the fractions eluting at 140 mM and 400 mM phosphate, and the total yield was greater than 95%. The fraction eluting at 140 mM phosphate was concentrated to dryness by centrifugal lyophilization, resuspended in 3 ml of sterile glass distilled water, and desalted on a column (1.5 by 20 cm) of Sephadex G15 equilibrated with 1 mM EDTA (pH 8.0). DNA was eluted in the void volume and concentrated to a volume of 2 ml by centrifugal lyophilization. Aliquots of 0.5 ml were layered on 5 to 30% sucrose gradients with a 0.25-ml cushion of 50% sucrose and centrifuged at 42,000 rpm in an SW50.1 rotor to an \( w^t \) value of 55,000. The sucrose gradients were fractionated from the bottom into three fractions; the lower 2.5 ml contained \( \lambda h80C,857dargF \) light strand, the next 0.5 ml contained no DNA, and the upper 2.5 ml of gradient contained \( \lambda h80C,857 I \) strand 4 to 5S fragments. Fractions containing \( \lambda h80C,857dargF \) light strand were pooled, 250 \( \mu g \) of poly(A) carrier was added, and the solution was concentrated to a volume of 7 ml by centrifugal lyophilization, desalted as described, and again concentrated to a volume of 3 ml. The sample was stored at 4°C over a drop of chloroform with a final yield of 77%. Fractions from the upper 40% of the gradients were pooled, concentrated by centrifugal lyophilization, desalted, and reconcentrated as described to yield 88% of the fragments originally used in the experiment. The fraction eluting in 400 mM phosphate was concentrated, desalted, and concentrated as described. Aliquots of 0.5 ml were layered on a 5 to 30% alkaline sucrose gradient (0.5 M NaCl, 0.2 M NaOH, 1 mM EDTA) with a 0.25-ml cushion of 50% sucrose and centrifuged at 42,000 rpm at 4°C in an SW50.1 rotor to an \( w^t \) value of 55,000. The lower 2.5 ml of the gradients was pooled and neutralized with 1.0 N HCl, and 250 \( \mu g \) of poly A carrier was added, and the mixture was concentrated, desalted, and concentrated once more as described to give a final recovery of 82% of \( \lambda h80C,857dargF \) strand. The individual resolved strands were unit length as demonstrated in Fig. 5 in which the sedimentation profile of \( \lambda h80C,857dargF \) freshly prepared by phenol extraction (Fig. 5a), after resolution of the \( I \) strand from the 140 mM fraction (Fig. 5b), and after resolution of the \( r \) strand from the 400 mM fraction (Fig. 5c) are compared by sedimentation through a 5 to 30% alkaline sucrose gradient (0.5 M NaCl-0.2 M NaOH).

Each of the resolved fractions was self-annealed for 16 h at 68°C in 0.2 M NaCl to permit any contaminating complementary strand to anneal; the phosphate concentration was adjusted to 140 mM, and each fraction was chromatographed on hydroxypatite at 60°C. More than 98% of the DNA passed through the column, indicating that there was insignificant cross-contamination with the complementary strand. A sample of the single-stranded DNA self-annealed as described was sedimented through an alkaline sucrose gradient as described (Fig. 5d). In view of the considerable thermal shearing obtained after self-annealing at 68°C and because of negligible cross-contamination of one resolved strand with the other, this step of the preparation procedure was usually omitted.

We have performed two experiments to confirm that the 140 and 400 mM fractions indeed constitute resolved strands of \( \lambda h80C,857dargF \).

(i) Self- and cross-hybridization. When
\( \lambda h80C,857dargF \) and \( r \) strand DNA were incubated separately at 68 C in 140 mM phosphate buffer at a concentration of 0.5 \( \mu \)g of DNA/ml, there was no conversion to a double-stranded form detectable by chromatography on hydroxyapatite; however, a mixture containing equal proportions of both strands renatured with second-order kinetics (Fig. 6).

(ii) Hybridization of \( \arg F \) mRNA to \( \lambda h80C,857dargF \) \( r \) and \( l \) strands. Purified mRNA was prepared from a strain making constitutive levels of \( \arg F \) message and ornithine transcarbamylase as described. Six aliquots, each comprising \( 3.05 \times 10^8 \) counts/min of \([^3H]\)uridine-labeled RNA, were hybridized to 5 \( \mu \)g of denatured \( \lambda h80C,857dargF \) DNA immobilized on a nitrocellulose filter. One filter was washed, and bound radioactivity was counted in Ready-Solv IV; RNA was eluted from the other filters as described by Bačevre and Szybal'ski (2). Approximately 0.2\% of the RNA input was recovered from specific hybridization to denatured DNA isolated from \( \lambda h80C,857dargF \) (Table 3). In contrast to these data, when mRNA was prepared under conditions of physiological repression from CA8000, a strain carrying the \( \arg R^+ \) allele, 0.06\% of the RNA input was found to hybridize specifically to DNA isolated from \( \lambda h80C,857dargF \) (Table 4). The purity of \( \arg F \) mRNA isolated under conditions of constitutive synthesis was demonstrated by rehybridization to denatured \( \lambda h80C,857dargF \) DNA and to \( \phi 80 \) DNA immobilized on nitrocellulose filters. It was found that 93\% of this input radioactivity bound specifically to \( \arg F \)-containing DNA, whereas only 2\% bound to \( \phi 80 \) DNA. Hybridization of 1,400 counts/min of this purified \( \arg F \) mRNA to 0.33 \( \mu \)g of \( \lambda h80C,857dargF \) \( r \) strand and \( l \) strand, isolated as described, resulted in 76\% of the input radioactivity hybridizing specifically to the \( r \) strand and 9\% to the \( l \) strand (Table 5).

**DISCUSSION**

The results presented in this work show that it is possible to separate the strands of the specialized transducing bacteriophage \( \lambda h80C,857dargF \) utilizing a procedure which takes no cognizance of the specific parts of the molecules which are either present or deleted. It is, therefore, possible to resolve the strands of any deletion or substitution mutant of \( \lambda \) or \( \phi 80 \)

![Fig. 6. Reassociation kinetics of \( l \) and \( r \) strand \( \lambda h80C,dargF \) DNA. Hybridization solutions contained a total of 2.5 \( \mu \)g of \( l \) strand DNA ( ), \( r \) strand DNA ( ), or a mixture of equal proportion of \( l \) and \( r \) strand DNA ( ) in 2.5 ml of 140 mM phosphate buffer-0.4\% sodium dodecyl sulfate. After various times of incubation at 68 C, samples (0.250 ml) were diluted into 2.5 ml of 140 mM phosphate buffer-0.4\% sodium dodecyl sulfate, and the quantity of single-stranded and native DNA was determined by chromatography on hydroxyapatite.](http://jvi.asm.org/)

**TABLE 3. Purification of \( \arg F \) mRNA isolated from EJ107**

<table>
<thead>
<tr>
<th>Total RNA input (( ^3H ) counts/min)</th>
<th>( ^3H ) counts/min bound to:</th>
<th>( ^3H ) counts/min bound to:</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>( \arg F ) DNA</td>
<td>( \phi 80 ) DNA</td>
</tr>
<tr>
<td>3,054,800</td>
<td>6,210</td>
<td>227</td>
</tr>
</tbody>
</table>

**TABLE 4. Isolation of \( \arg F \) mRNA from CA8000 grown in the presence of 100 \( \mu \)g of arginine per ml**

<table>
<thead>
<tr>
<th>Total RNA input</th>
<th>( ^3H ) counts/min bound to:</th>
<th>( ^3H ) counts/min bound to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \arg F ) DNA</td>
<td>( \phi 80 ) DNA</td>
</tr>
<tr>
<td>901,000</td>
<td>564</td>
<td>49</td>
</tr>
</tbody>
</table>

**TABLE 5. Hybridization of purified \( \arg F \) mRNA to \( \arg F \) and \( \phi 80 \) DNA**

<table>
<thead>
<tr>
<th>Purified ( \arg F ) mRNA ( ^3H ) counts/min input</th>
<th>( ^3H ) counts/min bound to:</th>
<th>( ^3H ) counts/min bound to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \arg F ) DNA</td>
<td>( \phi 80 ) DNA</td>
</tr>
<tr>
<td>1,400</td>
<td>1,290</td>
<td>30</td>
</tr>
</tbody>
</table>
by use of the technique described using single-stranded fragments with an S value of between 4 and 5 derived from one or more lambda phages to provide the maximum homology between the fragments and the genome to be resolved. The results obtained using λCb2 fragments to resolve a λh80 hybrid phage indicated that the choice of fragments isolated from another source should be more efficacious. The use of resolved fragments of λh80C,857 or a mixture of λCb2 and φ80 fragments was found to provide a satisfactory solution to the problem. An important aspect of this work is the applicability of the procedure we have described for the resolution of the DNA strands of specific DNA species obtained after digestion with the endonuclease Eco R1 of DNA isolated from a specialized transducing bacteriophage. The only modification required for this purpose is the sucrose gradient centrifugation step used for the separation of the resolved restricted DNA strand from excess single-stranded fragments.

It is possible that duplex DNA comprising unit length λh80C,857dargF r strand and λh80C,857 l strand fragments could be separated from excess single-stranded fragments and λh80C,857dargF l strand by centrifugation to equilibrium in a 1.7250-density cesium chloride density gradient; however, the use of chromatography on hydroxyapatite is much more rapid, considerably less expensive, and undoubtedly leads to a cleaner separation.

The data presented in Table 4 clearly indicate that control of the argF operon in the arginine biosynthetic regulon is effected, at least in part, at the transcriptional level. This is consistent with data presented by Pouwels et al. (15) for the argECBH genes of the arginine regulon. These workers demonstrated that argECBH mRNA levels were between 0.02% and 0.036% under conditions of physiological repression compared to a constitutive level of 0.23 to 0.42% of the total RNA synthesized in a 50-s pulse of [3H]uridine as judged by studies of mRNA hybridized to DNA isolated from a φ80 transducing phage carrying the genes ppc and argECBH. It is clear that synthesis of mRNA from bacterial operons neighboring argF is observed under conditions of physiological derepression of the arginine biosynthetic regulon; however, the data shown in Table 4 indicate that at least 70% of the mRNA, synthesized in a strain carrying the arg R- allele, which binds specifically to DNA isolated from the phage λh80C,857dargF is indeed argF mRNA. This is entirely consistent with the observation (Table 5) that 76% of purified argF mRNA binds specifically with λh80C,857dargF r strand DNA and clearly indicates that the λh80C,857dargF r strand carries sense information for the argF gene.

ACKNOWLEDGMENTS

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