A Specialized Transducing Phage, λ psrIA, for the Sorbitol Phosphotransferase of *Escherichia coli* K12

By A. BORONAT, M. C. JONES-MORTIMER* AND H. L. KORNBERG

Department of Biochemistry, University of Cambridge, Tennis Court Road,
Cambridge CB2 1QW, U.K.

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A specialized transducing phage for the *srlA* gene, specifying the sorbitol-specific Enzyme II of the phosphoenolpyruvate:sugar phosphotransferase system, was constructed and its DNA was analysed by restriction endonuclease digestion. Phage construction involved four steps: (1) integration of λ into the *srlA* gene; (2) selection of phage carrying (a) the left and (b) the right end of the *srlA* gene by means independent of the function of the new DNA acquired; (3) reconstitution of the *srlA* gene in a dilysogen of these two phage; and (4) the excision, using the heteroimmune lambdoid phage 21, of a plaque-forming *srlA*⁺ phage from the dilysogenic chromosome. Comparison of the DNA restriction digests of the transducing phage with those of its parents and of wild-type λ revealed fragments consisting partly of λ and partly of *Escherichia coli* DNA. The junction points in the intermediate phage define a site that must lie within the reconstituted gene of the final phage. This technique should be of general application in relating genes, cloned by our method, to DNA sequences.

INTRODUCTION

Most of the techniques described for the isolation of specialized transducing phage rely on the positioning of the gene to be cloned adjacent to an attachment site of a lambdoid phage (Morse *et al.*, 1956; Beckwith *et al.*, 1966; Press *et al.*, 1971; Shimada *et al.*, 1972). However, Davis & Calvo (1977) and Mata *et al.* (1978) have obtained specialized transducing phage for genes into which λ has integrated.

Because of our interest in the exclusion by glucose of other carbohydrates (Kornberg *et al.*, 1980), we wished to investigate more closely one of the membrane-bound Enzymes II of the phosphoenolpyruvate-dependent sugar phosphotransferase system, since we believe (Amaral & Kornberg, 1975) that these proteins are the sites at which catabolite inhibition (McGinnis & Paigen, 1969) may act. Since McEntee (1977) has shown that λ will integrate into *srlA*, the gene for the sorbitol Enzyme II, this appeared to be a suitable system for the production of specialized transducing phage.

METHODS

The *Escherichia coli* strains used are listed in Table 1; the phage strains are in Table 2. The general microbiological methods were those used previously (Jones-Mortimer & Kornberg, 1976). λ methods and media were those of Gottesman & Yarmolinsky (1968*a*) and Shimada *et al.* (1972).

λDNA was prepared by the rapid lysis technique of Davies *et al.* (1980). Digestions with *Eco*RI (BCL, London), *Hind*III, *Bam*HI and *Kpn*I (Bethesda Research Laboratories, Cambridge) were carried out in the buffers recommended by the manufacturers, using about 1 µg DNA in a final volume of 50 µl. Double digestions were performed by digesting first with the enzyme requiring the lower ionic strength and subsequently adjusting for the conditions required for the second enzyme. After addition of 10 µl of sample buffer [40 mM-EDTA, 0.1% (w/v) sodium dodecyl sulphate, 30% (w/v) Ficoll, 0.25% (w/v) bromophenol blue], samples were heated at 60 °C for

Table 1. *Strains of Escherichia coli used*

Strain	Relevant genotype	Source or reference
CSH25	<i>tyrT</i>	P. Oliver
H50	(<i>srl recA</i>) ^Δ <i>mtl</i>	P. Oliver
JM759	<i>tonA</i> (<i>gal uvrB</i>) ^Δ <i>ptsF ptsM</i>	Jones-Mortimer & Kornberg (1976)
JM1099	<i>galK</i>	Henderson <i>et al.</i> (1977)
JM1521	<i>tonA</i> ⁺ (<i>gal uvrB</i>) ^Δ <i>ptsF ptsM srlC</i>	JM759
JM1551	<i>srlA</i> :: <i>λcI857S7</i>	JM1521
JM1554	<i>srlA</i> :: <i>λcI857S7</i> <i>λ</i> ^R	JM1551
JM1684	(<i>λ</i> JM19) (<i>λ</i> JM20)	H50
JM1700	<i>srlA tyrT</i> (21)	*
JM1710	(<i>srl recA</i>) ^Δ <i>mtl</i> (<i>λc</i> ⁺)	*
JM1719	(<i>gal uvrB</i>) ^Δ <i>ptsF ptsM srlC</i> ⁺ <i>srlA</i>	*
RB341	<i>tyrT</i> (P2)	Buxton <i>et al.</i> (1978)
P4X	<i>tonA</i> ⁺	
PO345	<i>srl mtl</i>	P. Oliver

* A full description of the construction of these strains will be provided on request.

Table 2. *Strains of bacteriophage used*

Strain	Source
<i>λcI857S7</i>	P. Oliver
<i>λcIb2</i>	P. Oliver
<i>λch80del9</i>	P. Oliver
<i>λ</i> JM19 (Spi ⁻)	<i>E. coli</i> JM1554
<i>λ</i> JM20 (EDTA ^R)	<i>E. coli</i> JM1554
<i>λ</i> JM23 (<i>srlA</i> ⁺)	<i>E. coli</i> JM1684
<i>λ</i> JM24 (<i>srlA</i> ⁺)	<i>E. coli</i> JM1684
P1kc	M. D. Yudkin
21	P. Oliver

5 min and applied to horizontal agarose gels (0.5–1%, w/v) containing 40 mM-Tris/acetate buffer, pH 7.7, 1 mM-EDTA and 0.5 µg ethidium bromide ml⁻¹. Similar Tris/acetate/EDTA buffer was used in the electrode wells. Gels were run at 2–5 V cm⁻¹ and photographed under short wavelength u.v. illumination. Digests of *λcI857S7* DNA with *EcoRI* and *HindIII* were used as standards in all gels to relate mobility to fragment size, and as Fig. 3 indicates, all samples were internally calibrated.

RESULTS

Construction of λpsrlA⁺

Strain JM1521 was obtained from strain JM759 (Jones-Mortimer & Kornberg, 1976; Henderson *et al.*, 1977) in two stages. Bacteriophage P1kc grown on strain P4X was used to transduce strain JM759 (*tonA*) to glucarate-positive phenotype (Robertson *et al.*, 1980). A *tonA*⁺ recombinant from this cross was plated on fructose minimal medium at 30 °C to select for a sorbitol constitutive (*srlC*) mutation (Jones-Mortimer & Kornberg, 1976). Subsequent stages of the construction are outlined in Fig. 1. An *srlC* strain, JM1521, was lysogenized with *λcI857S7* by the procedure of Shimada *et al.* (1972). After growth to allow phenotypic expression the cells were plated, together with *λcIb2* and *λch80del9*, on glycerol minimal medium containing 2-deoxy-2-amino-D-glucitol (10 mM). Under these conditions, only strains that are both lysogenic for *λ* and lack the sorbitol phosphotransferase can grow, since the *λ* strains kill any non-lysogens, and the 2-deoxy-2-amino-D-glucitol inhibits strains expressing sorbitol phosphotransferase activity (Jones-Mortimer & Kornberg, 1976). One such lysogen, strain JM1551, was chosen, since it could grow on carbon sources other than sorbitol that require *ptsH* and *ptsI* activity and, on heat-pulse curing (Shimada *et al.*, 1972), it regained the

Fig. 1. Schematic representation of the construction of $\lambda psrLA$: (i) wild-type λ ; (ii) $srlA^+ att\lambda^A$ host; (iii) lysogen with λ integrated into $srlA$; (iv) EDTA-resistant phage of the $\lambda pgal$ type; (v) Spi^- phage of the $\lambda pbio$ type; (vi) hypothetical intermediate resulting from an int - xis -mediated recombination between structures (iv) and (v); (vii) $srlA^A att\lambda^+$ host; (viii) dilysogen with structure (vi) integrated at $att\lambda$; (ix) $\lambda psrLA$, the product of Ter-mediated excision through m and m' .

λ (i) was integrated into the $srlA$ gene of the $att\lambda^A$ host (ii) to yield the lysogen (iii). Induction and screening of the liberated phage yielded the phage (iv) and (v) each of which carries part of the $srlA$ gene. Simultaneous infection of a $srlA$ -deletion strain (vii) with (iv) and (v) yielded the Srl^+ tandem dilysogen (viii) perhaps via the hypothetical intermediate (vi). The sorbitol transducing phage (ix) were obtained from the dilysogen by Ter-mediated excision using phage 21.

ability to grow with sorbitol as carbon source. From this strain a λ^R derivative strain JM1554 was isolated by selection for resistance to λvir .

Strain JM1554 was thermally induced. The cells were then concentrated 10-fold by centrifugation and lysed by the addition of chloroform. After the removal of cell debris by centrifugation, portions of the supernatant fluid were plated on the P2 lysogen RB341. A Spi^- plaque, strain $\lambda JM19$ (Table 2), was purified for subsequent experiments. [Spi^- λ mutants have lost the *red* and *gam* genes and have acquired a recombinational hot-spot. Thus, when obtained from an induced lysate, they usually carry *E. coli* DNA (Lindahl *et al.*, 1970).] Other portions of the supernatant fluid were treated with EDTA (Gottesman, cited in Buxton *et al.*, 1978). A survivor of this treatment, strain $\lambda JM20$, was obtained as a plaque on *E. coli* strain CSH25. It was shown to be able to transduce the Srl^- *E. coli* strain PO345 to Srl^+ .

The two λ strains thus obtained were used to infect the *srlA recA* deletion strain H50, and progeny were selected on sorbitol minimal medium. One of these strains, JM1684, was apparently a dilysogen carrying both $\lambda JM19$ and $\lambda JM20$ integrated at *att λ* since it had the following properties: (1) non-lysogens obtained from it by heat-pulse curing were sorbitol-negative; (2) a thermally induced lysate transduced a *galK* strain (JM1099) to Gal^+ at a frequency of 1×10^{-7} ; (3) the lysate transduced strain H50 to Srl^+ .

A plot of the logarithm of the number of transductants obtained in this cross against the logarithm of the phage dilution employed had a slope of -1.2 , showing that many of the transductants must have arisen from infection by a single transducing particle. However, none of the transductants examined yielded plaque-forming transducing phage on induction; in contrast, such phage could be obtained from strain JM1684 by Ter-mediated excision (Gottesman & Yarmolinsky, 1968*b*; Mousset & Thomas, 1968). [In Ter-mediated excision the superinfecting phage mistakes the two tandem *cos* sites (*mm'* in Fig. 1) of the dilysogen for the equivalent sites in the replicative form of its own DNA and packages the DNA between these sites.]

Phage 21, which has the same host range as λ but differs from λ in the specificity of its N protein and of its *int* and *xis* proteins as well as in its immunity, was propagated on strain JM1684 at 30 °C in liquid culture. The lysate was plated at suitable dilutions on strain JM1700 [*srlA tyrT* (21)] on tetrazolium sorbitol medium at 30 °C. After incubation for 3 d, about 1% of the plaques had pale pink edges which indicated that the unlysed but infected cells round the edges could utilize the sorbitol in the medium. Two phages of λ immunity, $\lambda JM23$ and $\lambda JM24$, were isolated from one such plaque.

Characterization of $\lambda srlA^+$

Biological characterization. Both $\lambda JM23$ and $\lambda JM24$ transduced strain H50 to Srl^+ with $\lambda CI857S7$ as helper. That $\lambda JM23$ carried the complete *srlA*⁺ gene was shown by infecting strain JM1710 [(*recA srlA*)^A (λ^+)] in liquid culture. The results of this experiment are shown in Fig. 2. Neither $\lambda JM19$ nor $\lambda JM20$ stimulated the growth of strain JM1710 on sorbitol. A mixture of the two phage preparations was equally ineffective. Nevertheless, infection with $\lambda JM23$ allowed growth in the presence of sorbitol (but not in the absence of a carbon source). Since strain JM1710 is a recombination-deficient homoimmune lysogen, the *srlA*⁺ gene on the superinfecting phage must be expressed.

$\lambda JM23$ was also used to infect strain JM1719 (*srlA srlC*⁺ *ptsF ptsM att λ* ^A) and sorbitol-positive transductants were selected; these were fructose-negative and thermosensitive (but readily yielded fructose-positive derivatives). One such sorbitol-positive strain was streaked out for single colonies. Of 80 such colonies tested, 2 were found now to be sorbitol-negative and 2 were fructose-positive. We have previously shown (Jones-Mortimer & Kornberg, 1976) that in appropriate strains fructose can be utilized by the sorbitol phosphotransferase, if that system is expressed constitutively; our results are therefore consistent with the segregation of haploids from a *srlA srlC*⁺/ $\lambda srlA^+$ *srlC* diploid if both *srlA*⁺

Fig. 2. Effect of *λpsrIA* (*λJM23*) on sorbitol utilization by a sorbitol-negative strain. A maltose-grown culture of strain JM1710 [*(srlA recA)^A (λ^{c+})*] was incubated with maltose (■), sorbitol (▲) or no carbon source (△), or infected with *λJM23*, *λJM19*, *λJM20* or a mixture of *λJM19* and *λJM20* and incubated with sorbitol (●) or no carbon source (○). The increase in turbidity is shown as a function of time.

Fig. 3. Physical maps of *λpsrIA* and its parental phages. *EcoRI*, *HindIII*, *BamHI* and *KpnI* restriction sites are indicated as E, H, B and K, respectively. The *λ* attachment site (*att*) is indicated for *λcI857S7* and the maps are aligned through this site. The *E. coli* DNA is indicated as hatched boxes.

and *srlC⁺* (the inducible allele) are dominant. This supports our previous model of negative control of the *srl* operon and contrasts with the views of Lengeler (1975) and McEntee (1977) who favour positive control models. Either the gene we refer to as *srlC* is not identical to their *gutC*, or we are examining a situation that is the converse of that described by Markowitz & Rosenbaum (1965), who described a control system in which the exogenotic allele of a regulatory gene was always dominant.

Biochemical characterization. Restriction endonuclease analyses of the genomes of *λ* wild-type (*λcI857S7*), *λJM19*, *λJM20*, *λJM23* and *λJM24* were carried out using *EcoRI*,

*Hind*III, *Bam*HI and *Kpn*I. The physical maps constructed are shown in Fig. 3. The positions of the cleavage sites in λ wild-type are those described by Davies *et al.* (1980). As compared with λ wild-type, the *Bam*HI and *Eco*RI restriction sites to the right of the λ attachment site (*att*) are not present in λ JM19, thus indicating a replacement of λ DNA by *E. coli* DNA at this position. Similarly, the loss in λ JM20 of the *Eco*RI, *Hind*III and *Bam*HI restriction sites to the left of *att* indicates that the cloned DNA is replacing the λb region. The physical maps of λ JM23 and λ JM24, which carry the complete *srlA*⁺ gene, are consistent with the joining of the two parts of the *srlA* gene carried by λ JM19 and λ JM20.

The DNA of λ JM20, λ JM23 and λ JM24 has an extra *Kpn*I restriction site within the bacterial DNA inserted at the left of *att*. No *Eco*RI, *Hind*III or *Bam*HI restriction site has been detected in the cloned *E. coli* DNA.

The sizes of λ JM19, λ JM20, λ JM23 and λ JM24 DNA calculated from the sizes of the restriction fragments are 47.8, 43.8, 47.5 and 42.6 kilobase-pairs, respectively.

DISCUSSION

We have described a method for the isolation of a specialized transducing phage for the gene *srlA*, which specifies the membrane-bound Enzyme II of the phosphoenolpyruvate-dependent phosphotransferase system (Kundig *et al.*, 1964) that is specific for sorbitol. The rationale for the method was as follows (Fig. 1): (1) to integrate λ into the *srlA* gene; (2) to isolate aberrantly excised λ from this lysogen, selecting only on the basis of properties of the phages, not on the nature of the *E. coli* DNA carried by them; (3) to reconstitute the *srlA*⁺ gene from the two phages in a dilysogen; and (4) to isolate from the dilysogen a single phage carrying the intact *srlA*⁺ gene.

As a technique the method has two advantages. First, the selection of the initial phages does not rely on the genetic information of the *E. coli* DNA acquired. Secondly, comparison of the restriction maps of the phages (Fig. 3) indicates the presence of restriction fragments, peculiar to λ JM19 and λ JM20, that carry the left and the right sides of the λatt site, respectively. If the DNA of these fragments were sequenced, one would observe an unknown (*E. coli*) sequence attached to a known (λ) sequence. From the method of construction of the phage, these points defined in the DNA sequence must lie within the gene defined by the original λ insertion mutation, thus relating the two.

We do not as yet have a good estimate of the size of the *E. coli* DNA in our phages: the *E. coli* DNA in λ JM24 is between 3.4 and 9.1 kilobase-pairs. A more precise determination might be possible with a different restriction endonuclease, or alternatively by heteroduplex analysis.

The right-hand end of the DNA of λ JM23 is unlike that of λ JM24, λ JM20 or wild-type λ (Fig. 3), yet both λ JM23 and λ JM24 were isolated from the same lysogen. λ JM23 cannot have arisen by recombination between a phage of λ JM24 type and the phage 21 used in its isolation since the restriction map of phage 21 is quite different (data not shown). A more plausible explanation is that λ JM23 arose by recombination with a cryptic lambdoid prophage present in one of our strains, though this hypothesis would be difficult to test.

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Dedicated to the memory of Mary Randle Lunt.

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