Characterization of Lambda *cysB* and of Two Derivatives Carrying *cysB* Amber Mutations

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The specialized transducing phage λ *cysB* (Borck *et al.*, 1976) was found to carry about 5 kilobases of *Escherichia coli* DNA. It was shown to have an intact *cysB* gene but none of the known neighbouring genetic loci. The phage (which is known to be deficient in its site-specific recombination functions) was shown to integrate into the chromosome of bacterial recipients at the *cysB* locus. Excision from this site occasionally generated recombinant phages that had exchanged their *cysB* allele for the one originally present in the host. In this way λ *cysB* derivatives were prepared from lysogens of two strains carrying the amber mutations *cysB242* and *cysB257*; these phages were proved by several tests to contain the expected *cysB* amber mutations.

INTRODUCTION

In the enteric bacteria *Salmonella typhimurium* and *Escherichia coli*, cysteine biosynthesis is a key process in the incorporation of inorganic sulphur into organic molecules. The various steps in the pathway by which inorganic sulphate is assimilated and reduced to sulphide (a direct precursor of cysteine) are catalysed by the products of at least seven structural genes which lie scattered around the bacterial chromosome (for a review, see Smith, 1971). Expression of these genes is positively controlled by the product of the *cysB* gene in the presence of *O*-acetyl-L-serine (Jones-Mortimer, 1968). *cysB*, which is near min 28 of the standard *E. coli* map, comprises a single cistron (Tully & Yudkin, 1977), the product of which is known to be a protein (Tully & Yudkin, 1975).

Borck *et al.* (1976) constructed *in vitro* a specialized transducing derivative of bacteriophage lambda (λ *cysB*), by replacing the central *EcoRI* fragment of a lambda vector that contained only two functional target sites for this endonuclease with a fragment of *E. coli* DNA. The transducing phage was selected for its ability to complement an *E. coli cysB* point mutant host and was assumed to carry at least part of the *cysB* gene. It had, however, lost the phage functions that lie between lambda sites 1 and 3 for the *EcoRI* endonuclease. Since these include the *attP* site, *int*, *xis* and *redA*, λ *cysB* is expected to be deficient in site-specific and general recombination functions.

We now report some of the properties of this transducing phage and the preparation of two derivatives, each carrying a known *cysB* amber mutation.

METHODS

Bacteria and bacteriophages. All the *E. coli* strains used were K12 derivatives; they are described in Table 1. A non-lysogenizing mutant of phage P1 (P1 clear) was used throughout. The specialized transducing

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Table 1. *Bacterial strains*All strains were derivatives of *E. coli* K12 of mating type F⁻.

Strain	Genotype	Source, reference or derivation
W3110	Prototrophic	This laboratory
W1485	Prototrophic <i>supE</i>	This laboratory
MY306	<i>thr arg his cysB</i> – <i>supX</i> ^{del100} <i>strA</i>	This laboratory
HVT5	<i>thr arg his tonB</i> – <i>trp</i> ^{del} <i>strA supE</i>	H. V. Taylor
CA275	<i>lac</i> ^{am} <i>trp</i> ^{am} <i>supF</i>	M. R. Lunt
JT32	W3110 <i>pyrF</i> 287 <i>trpE</i> 9829	Tully & Yudkin (1977)
JT42	W3110 <i>cysB</i> 242 <i>trpE</i> 9829	Tully & Yudkin (1977)
JT57	W3110 <i>cysB</i> 257 <i>trpE</i> 9829	Tully & Yudkin (1977)
JT61	W3110 <i>cysB</i> 261 <i>trpE</i> 9829	Tully & Yudkin (1977)
JT79	W3110 <i>cysB</i> 279 <i>trpE</i> 9829	Tully & Yudkin (1977)
DM600	W3110 <i>cysB</i> – <i>supX</i> ^{del110}	Spontaneous <i>supX</i> deletion
DM601	W3110 <i>cysB</i> – <i>supX</i> ^{del110} <i>trpE</i> 9829	JT32 × P1 grown on DM600
DM601E	W3110 <i>cysB</i> – <i>supX</i> ^{del110} <i>trpE</i> 9829 <i>supE</i>	DM601 × P1 grown on HVT5
DM5201F	W3110 <i>cysB</i> 279 <i>trpE</i> 9829 <i>supF</i>	JT79 × P1 grown on CA275
PR242	W3110 <i>cysB</i> 242 <i>trpE</i> 9829	This work
PR257	W3110 <i>cysB</i> 257 <i>trpE</i> 9829	This work

phage λ *cysB* [λ *srI*(1-2)^{del} *cysB* *gam*210 *cI*857 *srI*4⁰ *nin*5 *srI*5⁰] was kindly provided by Professor W. J. Brammar. T-M buffer. This contained 10 mM-MgSO₄ in 10 mM-Tris/HCl pH 7.5.

Media. These have been described (Yudkin, 1976).

Amplification of single plaques. Single plaques were transferred from agar plates to 1 ml T-M buffer containing 10⁷ cells of an overnight culture of strain W1485. This mixture was incubated at 40 °C for 20 min and then diluted with 5 ml pre-warmed L broth and shaken at 37 °C for 3 to 5 h. Cell debris was removed by centrifugation and chloroform was added.

Induction of λ cysB lysogens. Single colonies of the lysogen were grown in L broth at 30 °C to a density of about 2 × 10⁸ cells ml⁻¹. The cultures were then transferred to a water bath at 42 °C and incubated for 20 min, and finally shaken at 37 °C for 3 to 5 h until visible cell lysis occurred.

Preparation of high titre stocks of λ cysB. A derivative of strain MY306 lysogenic for the phage was thermally induced as described above. To 500 ml of lysate, 50 g polyethylene glycol 6000 and 14.6 g NaCl were slowly added with stirring, and then the mixture was centrifuged (3000 *g* for 30 min at 4 °C). The pellet was resuspended in 10 ml T-M buffer and caesium chloride was added to a final density of 1.48 g cm⁻³. This suspension was then centrifuged to equilibrium (21 h at 40000 rev. min⁻¹ in a Spinco SW50.1 rotor at 4 °C), the tubes were punctured and fractions were collected. Peak fractions containing the phage were pooled, dialysed against 2 × 1000 vol. T-M buffer and then stored at 4 °C. These stocks had titres ranging from 2 × 10¹⁰ to 2 × 10¹¹ ml⁻¹.

Estimation of spontaneous curing frequencies of lysogens. This was done as described by Shimada *et al.* (1972).

Selection of λ cysB lysogens. Single colonies of recipient bacteria were grown in tryptone maltose broth at 37 °C to a density of 2 × 10⁸ to 4 × 10⁸ cells ml⁻¹, then harvested by centrifugation and resuspended in T-M buffer at 10⁹ cells ml⁻¹. To 0.1 ml of this suspension, phage were added at multiplicities of infection ranging from 0.01 to 5 plaque-forming units (p.f.u.) per bacterium. The mixtures were incubated at 33 °C for 20 to 30 min and then plated directly on either (i) minimal agar medium lacking cysteine (for selecting Cys⁺ transductants) – these plates were scored after 48 to 72 h at 33 °C or (ii) tryptone agar medium seeded with 10⁹ λ *srI*(1-2)^{del} *srI*3⁰ *cI* *srI*4⁰ *srI*5⁰ – these plates were incubated overnight at 33 °C and replica-plated to maltose minimal agar plates (supplemented with the necessary amino acids) which were then incubated at 33 °C for 48 h. Most of the non-mucoid colonies which grew up on these plates were λ *cysB* lysogens.

Other genetic techniques. These have been described by Yudkin (1976).

RESULTS

Size of the transducing fragment

By determining the buoyant density of λ *cysB* relative to that of wild-type λ in the same caesium chloride gradient, we estimated that the λ *cysB* genome is about 16.5 % smaller than the wild-type genome. Given the size of the lambda vector used in the construction of λ *cysB*

in vitro, we calculated that the bacterial fragment in this transducing phage is about 5 kilobases in length; recent analysis with restriction endonucleases suggests a length of 5.1 ± 0.2 kilobases (D. M. Mascarenhas, unpublished results).

λ cysB lysogens and the site of prophage integration

λ *cysB* lysogens were selected either with the aid of a clear-plaque phage mutant or by transduction of a *cysB* recipient to Cys⁺ (see Methods). Unless selection was maintained by one of these two methods λ *cysB* lysogens lost the prophage during growth in broth (the curing frequency was about 2×10^{-2} per cell per generation as against 2×10^{-6} for λ *attP*⁺ *cI857* lysogens). λ *cysB* lysogens are easy to recognize by virtue of the temperature-sensitive *cI857* allele on the prophage; they are also immune to superinfection with λ *cI* but sensitive to λ *vir*.

In a typical experiment, λ *cysB* generated Cys⁺ transductants of point mutant *cysB* recipients at a frequency of about 10^{-4} per p.f.u. when multiplicities of infection ranging from 0.01 to 5 phage particles per bacterium were used. Not all of these transductants were lysogenic. The proportion of temperature-sensitive colonies in a transductant population varied from less than 5 % to more than 95 % for the different point mutants used, but was constant for a given point mutant.

During growth on tryptone agar at 41 °C these temperature-sensitive lysogens gave rise to rare non-lysogenic survivors (sensitive to superinfection with λ *cI*), some of which were Cys⁺. The occurrence of Cys⁺ non-lysogenic transductants, and of heat-stable survivors of lysogenic transductants, suggested that recombination of *cysB*⁺ from the phage on to the host could take place. One might then expect *cysB* recombinant phages to occur in lysates prepared from lysogenic λ *cysB* transductants (see below).

The site of integration of λ *cysB* lysogens was determined in an interrupted mating experiment. The *cysB* HfrH donor was lysogenic for λ *cysB* and we presumed that the time of entry of Cys⁺ reflected the site of integration of the prophage. The results showed that this site was transferred very soon after *trpE* (in a clockwise direction of transfer) and is consequently close to, if not identical with, the chromosomal *cysB* locus.

λ *cysB* also integrated readily into a small F' factor (F'123) that carries the region of the *E. coli* genome between *pyrF* and the *trp* genes (which includes *cysB*).

Transducing ability of λ cysB

A series of 62 strains with point mutations in *cysB* had previously been isolated in this laboratory and ordered into 16 deletion groups (Tully & Yudkin, 1977). λ *cysB* was able to transduce to Cys⁺ all 10 of the mutants from this collection that we tested, including representatives of the most *pyrF*-proximal and most *trpE*-proximal deletion groups.

This phage was also able to transduce to Cys⁺ two long *cysB* deletions which extend from the neighbouring gene *supX* through all of the *cysB* gene. Cys⁺ transductants of the strains carrying these deletions were invariably temperature-sensitive; heat-stable survivors selected from these transductants were invariably Cys⁻. We assume that, probably through lack of homology, λ *cysB* is unable to undergo a recombinational exchange of *cysB* alleles with these strains. However, because λ *cysB* can generate Cys⁺ transductants from *cysB*-deleted strains with which it cannot recombine, it must carry an intact *cysB* gene.

We showed that λ *cysB* does not transduce to wild-type strains carrying the mutations *pyrF*287 or *trpE*9829, and also that it does not contain a functional *opp* gene (Barak & Gilvarg, 1974). (We know that the order of genes in this region of the chromosome is *pyrF*-*cysB*-*supX*-*opp*-*trpE* since one of our *cysB*-*supX* deletions is *opp*⁺.) We failed in attempts to determine if λ *cysB* carries *supX*.

Table 2. *Effect of sup mutations on the transducing frequency of λ cysB and its derivatives*

The three λ phages were used to transduce strains carrying four *cysB* mutations, *cysB214*, *cysB239*, *cysB261* and *cysB279* (Tully & Yudkin, 1977) in *sup*⁰, *supD*, *supE* or *supF* backgrounds. The *sup* strains were constructed by transducing *cysB trpE*^{am9829} to Trp⁺ with phage P1 grown on *supD*, *supE* or *supF* donor strains from which all *trp* structural genes had been deleted. The isogenic *sup*⁰ strains were constructed by performing similar transductions with phage P1 grown on a *sup*⁰ *trp*⁺ strain. The number of Cys⁺ transductants obtained per 10⁷ donor λ phages (mean of two estimations) was used to compute the ratios given.

Transducing phage	Average ratio of transducing frequency		
	<i>supD/sup</i> ⁰	<i>supE/sup</i> ⁰	<i>supF/sup</i> ⁰
<i>λcysB</i> ⁺	1.7	1.0	2.2
<i>λcysB242</i>	17.0	8.0	21.0
<i>λcysB257</i>	17.0	9.5	21.0

λ cysB-amber derivatives

Derivatives of *λ cysB* carrying amber mutations *cysB242* and *cysB257* were isolated as follows. *λ cysB* lysogens of JT42 and JT57 were induced and the lysates were plated on W1485. Single plaques were picked and amplified in titre (see Methods). Each isolate was then tested for transducing ability on the respective *cysB* parent strain. Of about 35 isolates tested in this fashion, one isolate in each case was found to be unable to transduce the parental strain to Cys⁺. These two phages were named *λ cysB242* and *λ cysB257*, and were tentatively assumed to have acquired by recombination the *cysB* allele originally present in the host.

In *sup*⁰ strains *λ cysB242* and *λ cysB257* were able to transduce to Cys⁺ (albeit at substantially reduced frequencies) all point mutations within *cysB* tested except for the parental mutation. However, in *sup* strains they were able to transduce all recipients tested at frequencies comparable to those observed with *λ cysB*⁺. Table 2 shows that amber suppressors stimulated the recovery of transductants 8- to 22-fold in the case of *λ cysB242* and *λ cysB257* but not in the case of *λ cysB*⁺. This is the expected result if *λ cysB242* and *λ cysB257* harbour amber mutations within the *cysB* gene.

The two putative *cysB*-amber phages were also able to generate Cys⁺ transductants of the *cysB*-deleted *supE* strain DM601E but not of the isogenic *sup*⁰ strain DM600. *λ cysB*⁺, on the other hand, was able to transduce the two strains at similar frequencies. As the transducing phage cannot recombine with the long *cysB-supX* deletions in these strains, *cysB*-amber phages would be expected to supply a functional *cysB* protein only in the suppressing recipient.

Proof of the presence of the parental amber alleles in λ cysB242 and λ cysB257

Phage P1 was grown on a *λ cysB242* lysogen of MY306 (a strain that carries a deletion of the whole *cysB* gene) and used to transduce the *supF* strain DM5201F to Cys⁺ at 41 °C. Twelve transductants were purified and pooled, and phage P1 was grown on this pool of Cys⁺ transductants and used to transduce strain JT32 (*trpE cysB*⁺ *pyrF sup*⁰) to Pyr⁺. Some of the Pyr⁺ transductants were Cys⁻. The *cysB* allele present in them could only have been derived, given the genetic manipulations just described, from *λ cysB242*. One of these Cys⁻ isolates (which retained *trpE*) was numbered PR242.

Strain PR257 was constructed by an exactly similar series of steps starting from a *λ cysB257* lysogen of MY306.

If *λ cysB242* and *λ cysB257* do carry the mutations *cysB242* and *cysB257*, it follows that the newly constructed strains PR242 and PR257 should be identical to JT42 and JT57. We used two tests to establish whether this was so. First, the newly constructed strains, like the

Table 3. *Recombination frequency between three standard cysB mutations and the cysB mutations in strains JT42, PR242, JT57 and PR257*

Phage P1 grown on *trp*⁺ donors was used to transduce the *trp cysB* recipients. The results show the percentage of Cys⁺ colonies among the Trp⁺ transductants (the total number of which is given in parentheses). The *trp*⁺ donors for these experiments were constructed by transducing JT61, JT42 and JT57 to Trp⁺ with phage P1 grown on W3110.

Recipient strain	cysB mutation in donor		
	<i>cysB261</i>	<i>cysB242</i>	<i>cysB257</i>
JT42	0.9 (221)	0.0 (101)	4.0 (354)
PR242	0.7 (557)	0.0 (1142)	4.0 (475)
JT57	ND	6.8 (588)	0.0 (913)
PR257	ND	6.0 (1178)	0.0 (1107)

ND, Not determined.

original ones, were suppressed to Cys⁺ by lysogeny with ϕ 80*supF* (Russell *et al.*, 1970). Secondly, a series of three-point crosses with three *cysB* donors showed close similarities in the recombination frequencies obtained with PR242 and JT42, on the one hand, and PR257 and JT57, on the other (Table 3).

From all these results we conclude that λ cysB242 and λ cysB257 carry, respectively, the *cysB242* and *cysB257* amber alleles. We have also established that parental-type λ cysB⁺ phages can be recovered from λ cysB242 and λ cysB257 by recombination with suitable bacterial recipients.

DISCUSSION

From its method of construction λ cysB is known to be deficient in its site-specific and general recombination functions. It also lacks a functional *att* site. Consequently, we were not surprised to find that λ cysB integrates at the host *cysB* locus. It is likely that this integration occurs via the host recombination pathways which use the homology that exists between the *cysB* DNA fragment on the phage and the corresponding region of the chromosome. The reverse of this mechanism would lead to excision, and to the acquisition, by a certain fraction of the phage, of the host *cysB* allele.

Our use of this recombination mechanism to prepare λ cysB242 and λ cysB257 has given us a set of three phages, one containing the wild-type *cysB* gene and two containing amber mutations therein. By employing these phages we have managed to identify a 39000 dalton polypeptide as the product of the *E. coli cysB* gene (Mascarenhas & Yudkin, 1980).

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