Lambda Transducing Phage and Clones Carrying Genes of the *cysJIHDC* Gene Cluster of *Escherichia coli* K12

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DNA from each of two specialized transducing λ phage, λ dcysJIHD and λ cysJ, has been analysed by heteroduplex mapping. The segment of the *Escherichia coli* chromosome carried by λ dcysJIHD was shown to be large, approximately 18 kb in length, and to replace a large length of λ DNA, approximately 11 kb, which includes the genes for integration and recombination. Thus λ dcysJIHD is a *bio*-type transducing phage. λ cysJ was shown to have lost very little λ DNA and to carry about 8 kb of bacterial DNA. Sites for several restriction endonucleases were mapped in the DNA from each phage and cloning experiments located some of the genes of the cluster in relation to the restriction map. Cysteine regulation of the cloned *cysJ* and *cysD* genes was shown as well as cysteine regulation of β -galactosidase in some constructs. The direction of transcription of the *cysD* gene was established, and from physical evidence the size of the 'silent section' between the *cysH* and *cysD* genes was estimated to be at least 11 kb.

INTRODUCTION

The pathway of cysteine biosynthesis in *Escherichia coli* K12 and *Salmonella typhimurium* consists of two converging branches. In the sulphur branch, sulphate is ultimately reduced to sulphide and in the carbon branch, serine is acetylated to form *O*-acetylserine. In the final step, *O*-acetylserine reacts with sulphide to form cysteine (Trudinger & Loughlin, 1981; Umbarger, 1978; Siegel, 1975). The structural genes for the enzymes of cysteine biosynthesis are located in different regions around the chromosome. The genes for the sulphur branch enzymes form a cluster at 59 min in the *E. coli* linkage map (Bachmann, 1983) and at 60 min in the linkage map of *S. typhimurium* (Sanderson & Roth, 1983). Jones-Mortimer (1973) has shown that the orientation and gene order of this cluster in *E. coli* is: *lysA thyA argA cysJIHDC*. Studies of nonsense and deletion mutants of *S. typhimurium* have shown that three genes of this cluster, *cysJ*, *cysI* and *cysH*, form an operon which is transcribed from the *cysJ* gene end and that genes *cysD* and *cysC* do not form part of this operon (Loughlin, 1975, 1976).

A λ specialized transducing bacteriophage, λ dcysJIHD, carrying most of this cluster has been shown to be denser than λ in a CsCl gradient and to be a defective phage. DNA from this phage had been used to study the synthesis of cysteine messenger RNA (Fimmel & Loughlin, 1977). In order to study the cluster further, we have isolated additional deletion mutants and transducing phage. Here we report the map order of the end points of deletion mutants and transducing phage and characterization of λ dcysJIHD and another phage (λ cysJ) by heteroduplex and restriction endonuclease analysis. The location of some genes by cloning various fragments of the cluster is also described.

METHODS

Bacterial strains and vectors. The strains used were derived from *E. coli* K12 and are listed in Table 1. Plasmids used for cloning were pBR322 (Sutcliffe, 1979), pLG400 (Guarente *et al.*, 1980) and pMC1403 (Casadaban *et al.*, 1980).

Table 1. *E. coli* K12 strains

The following strains have been described by Fimmel & Loughlin (1977): CSH45, JM57, JM81A, JM221, RL144, RL145, RL149, RL155, X7150.

Strain	Genotype	Source or derivation
C600K	<i>galK</i> *	N. S. Willetts
KB9163	HfrKL16 <i>thi nalA900</i> †	K. D. Brown
MC4100	F ⁻ <i>araD139 Δ(argF-lac) U169 rpsL150 relA1 ffbB5301 deoC1 ptsF25 rbsR</i>	Casadaban (1976); See also Silhavy <i>et al.</i> (1984)
NK5031	<i>lacZΔ m5265</i> ‡	G. Zurawski
RL75	HfrKL16 <i>thi nalA900 (λcI857 Sam7 xisam6 b519 b515 nin5)</i> †§	Fimmel & Loughlin (1977)
RL76 and RL77	As for RL75	This work
RL147	HfrH $\Delta(cysJH)$ <i>str^s λ</i> †	Fimmel & Loughlin (1977)
RL157	HfrH <i>sup Δ(cysJH) (λcI857 Sam7) (λdcysJH)</i> †	A. L. Fimmel
RL198	F ⁻ <i>cysJ (λcI857 Sam7)</i>	RL144 lysogenized by <i>λcI857 Sam7</i>
RL363	<i>cysJ</i> [pRL5]*	This work
RL364	<i>lacZΔm5265</i> [pRL6]‡	This work
RL365	<i>cysJ</i> [pRL7]*	This work
RL367	<i>cysJ</i> [pRL9]*	This work
RL405	F ⁻ <i>cysJ (λcI857 Sam7) (λcysJI)</i>	This work
RL406	F ⁻ <i>cysJ (λcI857 Sam7) (λcysJ)</i>	This work
RL417	HfrKL16 <i>nalA900 thi Δ(cysJ)</i> †	This work
RL421	<i>cysJ</i> **¶	This work
RL428	<i>cysJ</i> **††	This work
RL432	<i>cysD</i> **‡‡	This work
RL439	<i>cysJ</i> [pRL98]**	This work
RL440	<i>cysJ</i> [pRL99]**	This work
RL441	<i>cysJ</i> [pRL100]**	This work
RL500	<i>cysJ</i> [pRL1]*	This work
RL502	<i>cysJ</i> [pRL3]*	This work
RL622	<i>cysD</i> [pRL5]**	This work §§
RL641	<i>lacZΔm5265</i> [pRL81]‡	This work
RR1	**	G. Zurawski

* Other markers F⁻ *thr-1 leu-6 thi-1 supE44 lacY1 tonA21 λ*⁻.

† Other markers $\Delta(gal-att^+-bio-uvrB)$.

‡ Other markers F⁻ *suIII⁺ nal^R*.

§ Prophage integrated near the *cysJ* gene.

|| Other markers: *thr-1 leu-6 trp-1 his-1 argH1 thi-1 xyl-7 ara-13 mtl-2 gal-6 lacY1 str-9 tonA2 supE*.

¶ Derived from strain C600 by selection of Thy⁻ using trimethoprim, then the *argA* marker was brought in from JM221 by P1 cotransduction, then the *cysJ* mutation brought in by P1 cotransduction from strain RL144.

** Other markers *proA2 leuB6 thi-1 lacY1 hsdR hsdM supE44 λ⁻ trpR55 ara-14 galK2 rpsL20 xyl-5*.

†† Derived from strain RR1 by the route described for strain RL421.

‡‡ Derived from strain RR1 by the route described for strain RL421 except that the *cysD* mutation was brought in from strain RL148 (an Arg⁺ derivative of JM221) by P1 cotransduction.

§§ Derived by transforming pRL5, obtained from strain RL363, into strain RL432.

Strain constructions and tests of lysates. Secondary site lysogens were isolated by lysogenization of strain KB9163 ($\Delta att\lambda$) with *λcI857 Sam7 xisam6 b519 b515 nin5* by the method of Shimada *et al.* (1972). Independent lysogens were grown at 30 °C in L broth and samples plated onto tryptone agar and incubated at 42 °C overnight. Temperature-resistant survivors were replica plated onto minimal agar to screen for the Cys⁻ phenotype. Lysogens giving Cys⁻ derivatives were used to prepare lysates by heat induction. These were tested for the ability to transduce a *cysJ* strain (RL144) to Cys⁺. Lysogens yielding such transductants thus had the prophage integrated near the *cysJ* gene.

Media and methods used for the isolation of deletion mutants and transducing phage have been described previously as have HFT (high frequency transduction) tests (Fimmel & Loughlin, 1977). The following methods were the same as those used previously except for the minor modifications mentioned. For phage assays, the host strain X7150 was grown in tryptone broth supplemented with maltose to 1% (w/v) and tryptophan to 40 µg ml⁻¹.

For transduction assays, recipient strains were grown in Kmal medium (Steege & Low, 1975). Phage lysates were prepared as before except that induced cells were harvested and resuspended in SM buffer (Miller, 1972) prior to lysis with chloroform. The method used for the preparation of *λ*c1857 *Sam7 xisam6 b519 b515 nin5* has been described by Ingle & Loughlin (1980).

Cross streaking tests. Recipient strains to be tested were patched to minimal plates supplemented with maltose to 0.2%. After growth of these patches, 0.05 ml donor lysate was streaked along a line across the width of a selection plate supplemented for control and test transductants. Cells of the test strains were streaked in a single movement along a line from top to bottom of the selection plate at right angles to the lysate line. Starved cells (Gottesman & Yarmolinsky, 1968) of RL144 served as the control recipient strain and were similarly cross streaked near each end of the lysate line. Recipient strains giving no growth above and growth below the lysate line were considered to be transduced by phage in the lysate.

P1 transductions. These were performed by the method of Miller (1972) except that adsorption mixtures comprised a total volume of 0.4 ml and 0.4 ml of 1 M-sodium citrate was added to the adsorption mixes prior to plating with 2.5 ml of F top agar (Miller, 1972).

Preparation of phage DNA. The methods used for phage purification and extraction of DNA have been reported by Fimmel & Loughlin (1977).

Heteroduplex analysis. Heteroduplex preparations were carried out using the formamide technique of Davis *et al.* (1971) and Davis & Parkinson (1971) as modified by G. D. Clark-Walker (personal communication). Analytical grade formamide for hypophase solutions was obtained from Ajax Chemicals or BDH. For hyperphase solutions the formamide was obtained from Mallinckrodt and cytochrome *c* from Sigma. Electron microscope copper grids (400 square mesh, 3.05 mm diam., from Polaron Equipment) were coated with Parlodion (Mallinckrodt) using redistilled *n*-amyl acetate (Polysciences). After picking up the DNA, the grids were rinsed with redistilled isopentane and shadowed with platinum/palladium (80:20 0.2 mm diam. wire from Polysciences) mounted on 1.0 mm diam. tungsten wire from Wright) then rotated through 90° and shadowed again. The grids were then viewed in a Philips 201C electron microscope at an accelerating voltage of 60 kV with a condenser aperture of 30 µm. Photographs were taken at magnifications of 7000× and 20000×. Photographic prints of heteroduplex DNA molecules were measured with a Talos digitizer linked to a Data General Eclipse 230Z computer or with a Leitz A.S.M. Semiautomatic Image Analyser.

Recombinant DNA techniques. Restriction endonucleases were obtained from Amersham, BRL, Boehringer Mannheim or New England Biolabs and used in accordance with the supplier's recommendations or with the buffers described by Maniatis *et al.* (1982). Digestion mixtures were incubated for 60–120 min at 37 °C for complete digestion. Partial digestion was achieved either by using insufficient enzyme or by incubating for shorter periods of time or by a combination of these conditions. Double digests were achieved by cleaving complete digests, described above, with a second restriction endonuclease. The buffer salt concentration was adjusted, if necessary, to suit the second enzyme and digestion mixtures were then incubated for a further 60–120 min. Reactions were stopped by the addition of a solution containing 4 M-urea, 50% (w/v) sucrose, 50 mM-EDTA and 0.1% bromophenol blue.

The DNA fragments were separated by electrophoresis in 0.7% agarose (Sigma, low electroendosmosis) slab gels, 3 mm thick, in a horizontal apparatus (model 800, Aquebogue Machine and Repair Shop) in 36 mM-Tris/30 mM-sodium phosphate buffer, pH 7.5, containing 10 mM-EDTA. Electrophoresis was done at a voltage gradient of 1.5–2 V cm⁻¹ with the gel submerged in the buffer. The gels were stained with ethidium bromide (2 µg ml⁻¹) for 30 min. The bands were visualized by illumination with a short wavelength transilluminator (model C61, UV Products) and photographed using Polaroid type 55 film or Kodak Tri-X Pan Professional film and a Kodak red filter.

Plasmid DNA was purified by the method of Selker *et al.* (1977). T4 DNA ligase was obtained from Boehringer Mannheim and used according to the supplier's recommendation. Transformation of cells was done using the procedures of Cohen *et al.* (1972) and Davis *et al.* (1980).

Plasmid constructions. Details of these are given in Table 2.

Enzyme assays. Cultures, grown usually overnight at 37 °C in minimal medium with djenkolic acid (0.42 mM) or cystine (0.42 mM) as sole sulphur source, were inoculated into the same medium and grown at 37 °C. Cells were usually harvested in late exponential phase.

For NADPH-cytochrome *c* reductase assays, cells were resuspended in 0.1 M-potassium phosphate buffer pH 7.7, containing 1 mM-EDTA, sonicated and the debris removed by centrifugation. Assays were done as described by Loughlin (1975). For ATP sulphurylase assays, frozen cells were disrupted in a modified Hughes press and the preparations mixed with 0.1 M-potassium phosphate buffer pH 7.7, containing 1 mM-EDTA, and debris removed by centrifugation. ATP sulphurylase assays were done by the method of Kredich (1971) with an extract of strain JM221 (*cysD*) as a source of adenosine phosphosulphate kinase and using phenol sulphokinase prepared from rabbit liver by method 1 of Gregory (1962).

For β-galactosidase assays, culture samples were toluenized and assays done as described by Miller (1972).

Soluble protein in cell-free extracts was measured by the method of Lowry.

Table 2. *Fragments, vectors and host strains used in plasmid constructions*

Plasmid	Fragment cloned			Vector used*	Host strain used in transformation†
	Source	Ends	Size (kb)		
pRL1	<i>λcysJ</i>	<i>Hind</i> III- <i>Bam</i> HI	11.9	pBR322	RL421
pRL3	pRL1	<i>Hind</i> III- <i>Sa</i> I	8.95	pBR322	RL421
pRL5	<i>λdcysJIHD</i>	<i>Bgl</i> II- <i>Bgl</i> II	5.43	pMC1403	RL421
pRL6	<i>λdcysJIHD</i>	<i>Bam</i> HI- <i>Bam</i> HI	3.80	pLG400	NK 5031
pRL7	<i>λdcysJIHD</i>	<i>Sma</i> I- <i>Sma</i> I	4.45	pMC1403	RL421
pRL9	<i>λdcysJIHD</i>	<i>Sma</i> I- <i>Sma</i> I	7.55	pMC1403	RL421
pRL81	<i>λdcysJIHD</i>	<i>Hind</i> III- <i>Hind</i> III	7.47	pLG400	NK 5031
pRL98	pRL9	<i>Bam</i> HI- <i>Bgl</i> II	4.21	pBR322	RL428
pRL99	pRL9	<i>Sph</i> I- <i>Hind</i> III	4.13	pBR322	RL428
pRL100	pRL9	<i>Sph</i> I- <i>Bgl</i> II	1.63	pBR322	RL428

* For each construction the vector was digested with the same enzyme(s) used for the cloned fragment except that *Bam*HI was used instead of *Bgl*II in digesting the vector for the constructions of pRL5, pRL98 and pRL100.

† For pRL1 and pRL3 Ap^R, Cys⁺ colonies were selected. For pRL98, pRL99 and pRL100, selection was for Ap^R and Cys⁺ colonies were detected after replica plating. For pRL5, pRL6, pRL7 and pRL9, selection was for Ap^R on plates containing djenkolic acid and Xgal (5-bromo-4-chloro-3-indolyl β-D-galactopyranoside). Blue colonies were chosen for further analysis.

RESULTS

Integration of λ near cysJ

Two secondary site lysogens, strains RL76 and RL77, were isolated by lysogenization of KB9163 with *λcI857 Sam7 xisam6 b519 b515 nin5*. Both were shown to have the λ prophage integrated near the *cysJ* gene by the isolation of transducing phage carrying genes from this region of the chromosome (see Methods).

Transducing phage

Lysates prepared from the secondary site lysogens were used to transduce a lysogen with a *cysJ* point mutation (strain RL198) to Cys⁺. Transductant colonies were purified and screened by HFT tests (see Methods) using *cysJ* (RL144) and *cysI* (JM57) mutant recipients. Patches giving transductants with the *cysJ* mutant but few or no transductants with the *cysI* mutant were used to prepare lysates which were assayed for their ability to transduce RL144 (*cysJ*), JM57 (*cysI*) and, for some, RL145 (*cysH*) to Cys⁺.

Three lysates transduced RL144 (*cysJ*) but not JM57 (*cysI*) to Cys⁺. One of the three strains yielding these lysates was selected for further study. This strain was designated RL406 and the transducing phage, which originated from RL76, was designated *λcysJ*. Several lysates transduced JM57 (*cysI*) but not RL145 (*cysH*) to Cys⁺. A strain giving one of these lysates was designated RL405 and the transducing phage, derived from RL77, was designated *λcysJI*.

Deletion mutants

Survivors of temperature induction at 42 °C of strain RL75 were screened for Cys⁻ derivatives by replica plating. Cys⁻ colonies were purified and used in cross streaking tests with a lysate containing *λcysJ*. Four strains were selected which yielded transductants in these cross streaking tests and transduction assays with *λcysJ* confirmed this result. All four strains could be transduced to Cys⁺ by P1 grown on RL144 as donor. One strain was selected and designated RL417.

Combination of the above results with the positions of the end points of *λdcysJIHD*, *λdcysJIH* and strain RL147 (Fimmel & Loughlin, 1977) and the order of genes in the cluster (Jones-Mortimer, 1973) enabled the establishment of the order of end points and mutations across the cluster (Fig. 1).

Fig. 1. Schematic diagram of the map order of point mutations and end points of deletion mutants and transducing phage. Horizontal lines represent DNA of the cluster present on transducing phage or in deletion mutants. Short vertical lines represent point mutations or the end points of deletions or bacterial DNA carried by the transducing phage. The last line represents the approximate positions of the genes of the cluster. These are not drawn to scale nor is the 'silent section' depicted (see Discussion). Due to this 'silent section', the novel joint of $\lambda dcysJIH$ may be located to the left of the novel joint of the deletion in strain RL147 [$\Delta(cysJIH)$].

Heteroduplex analysis

Heteroduplex molecules formed between $\lambda dcysJIHD$ DNA, prepared from strain RL155, and $\lambda cI857$ *Sam7* DNA, from strain CSH45, showed two deletion loops and one large substitution loop. Their structure is shown schematically in Fig. 2(a). The two deletion loops correspond in position to the *b519* and *b515* deletions present in the *b* region of the parent of the transducing phage ($\lambda cI857$ *Sam7 xisam6 b519 b515 nin5*). Thus the *b* region has not been lost in the formation of $\lambda dcysJIHD$ and as the substitution loop is large, the phage DNA lost must correspond to the genes for recombination to the right of the attachment site (Szybalski & Szybalski, 1979). Thus $\lambda dcysJIHD$ is a *bio*-type transducing phage. No evidence was found for a deletion loop due to the *nin5* deletion of the parent of $\lambda dcysJIHD$.

The length of H given in Fig. 2(a) was calculated using the double stranded length to the left of the attachment site ($A + C + E$) as an internal standard (Davis *et al.*, 1971; Davis & Parkinson, 1971; Lis & Schleif, 1975). The value of 22.5 kb for this length was derived from a value of 48.502 kb for λ DNA and the known locations of the attachment site (Sanger *et al.*, 1982) and the *b519* and *b515* deletions (Szybalski & Szybalski, 1979). The ratio of the double stranded region to the right of the attachment site (H) to the internal standard ($A + C + E$) was 0.44, corresponding to a value of 9.9 kb for the size of H. Of the two single stranded arms of the substitution loop, the longer (G) must correspond to bacterial DNA and the shorter (F) to λ DNA, as $\lambda dcysJIHD$ has a greater density than λ (Fimmel & Loughlin, 1977). Thus the shorter single stranded λ DNA of the substitution loop (F) is 10.9 kb and was used as a single stranded standard to measure the single stranded bacterial DNA (G) of the substitution loop, giving a value of about 18 kb.

A schematic representation of the heteroduplex molecules formed between $\lambda cysJ$ DNA and $\lambda cI857$ *Sam7* DNA is shown in Fig. 2(b). Of the three loops evident, two correspond to deletion loops due to the *b519* and *b515* deletions. The third loop is more likely to be an insertion loop due to the bacterial DNA carried by $\lambda cysJ$ rather than a substitution loop as the length

Fig. 2. Schematic diagram of heteroduplex molecules formed between *λcysJ* *Sam7* DNA and *λdcysJIHD* (a) or *λcysJ* (b) DNA. The mean values and standard deviations are calculated from 11 measured molecules for *λdcysJIHD* and 20 measured molecules for *λcysJ* and are given in kb.

Fig. 3. Locations of restriction sites in *λdcysJIHD* DNA and *λcysJ* DNA. Restriction site designations: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hind*III; Sa, *Sal*I; Sm, *Sma*I; Sp, *Sph*I. No site was found for *Sac*I or *Xba*I in *λcysJ* DNA neither was a site found for *Sac*I, *Sal*I or *Xba*I in *λdcysJIHD* DNA. The open box represents bacterial DNA and the shaded box *λ* DNA. The predicted orientation of the *cys* genes in *λdcysJIHD* DNA is shown below the map. The *cys* genes are not drawn to scale.

corresponding to F was detected in only three of the 20 heteroduplex molecules measured. In these three molecules, the length F may well be due to overlapping of the end of the single stranded loop and part of the double stranded DNA. The total of the single stranded deletion loops was used as an internal standard to gain an estimate of about 8 kb for the size of the bacterial insertion loop.

Fig. 4. Clones derived from *λcysJIHD* or *λcysJ* DNA. The bacterial DNA of each phage is shown with nearby *λ* sequences. Underneath each is shown the insert fragment for each clone. The open box represents bacterial DNA, the shaded box *λ* DNA and the line vector DNA. Restriction site designations are the same as shown in Fig. 3. Where the orientation of the insert with respect to the vector is unknown or where pBR322 was the vector used, the vector line is not shown. The arrows below the *lacZ* gene indicate the direction of transcription needed for expression. The name of the vector used in the construction of each clone is shown in parentheses. To the right is shown the letter designation of the *cys* mutation where the plasmid transforms that mutant to Cys⁺. — represents no Cys⁺ transformants detected.

Restriction mapping and cloning

DNA from two transducing phages, *λcysJIHD* and *λcysJ*, which each carry the *cysJ* gene, was mapped for the sites for several restriction endonucleases. From the left end of the bacterial DNA present in each phage, the first two sites were found to be in equivalent positions, namely *Sma*I site 2 and *Sph*I site 4. The third site in *λcysJIHD* DNA, namely *Bgl*II site 2, was not found in *λcysJ* DNA (see Fig. 3). From that site to the right end of the bacterial DNA in each phage the sites do not correspond.

Table 3. *Cysteine regulation of gene expression*

Strain	Relevant chromosomal mutation	Plasmid	Gene product assayed	Specific activity* for cells grown on:		Derepression ratio†
				Djenkolic acid (0.42 mM)	Cysteine (0.42 mM)	
RL367	<i>cysJ</i>	pRL9	<i>cysJ</i>	4700	69	68
RL421	<i>cysJ</i>	—	<i>cysJ</i>	0	0	—
RL622	<i>cysD</i>	pRL5	<i>cysD</i>	240	0	> 240
RL432	<i>cysD</i>	—	<i>cysD</i>	0	0	—
RL363		pRL5	<i>lacZ</i>	430	240	1.8
RL364	<i>lacZΔm5265</i>	pRL6	<i>lacZ</i>	3450	11	310
RL365		pRL7	<i>lacZ</i>	49	5	10
RL367		pRL9	<i>lacZ</i>	910	80	11
RL421		—	<i>lacZ</i>	5	4	1
MC4100	<i>ΔlacU169</i>	—	<i>lacZ</i>	0	0	—

* *cysJ*: NADPH-cytochrome *c* reductase. Specific activity given as nmol cytochrome *c* reduced min⁻¹ (mg protein)⁻¹. *cysD*: ATP sulphurylase. Specific activity given as nmol 3'-phospho-5'-adenyl sulphate (PAPS) produced h⁻¹ (mg protein)⁻¹. *lacZ*: units as defined by Miller (1972).

† Ratio of specific activity for cells grown on djenkolic acid to that for cells grown on cysteine.

To locate some of the genes on the bacterial DNA carried by *λdcysJIHD* or *λcysJ*, various fragments were cloned into the fusion vectors pLG400 and pMC1403 as well as into pBR322. The two fusion vectors were chosen in an attempt to detect cysteine regulated promoters. Such detection would only be possible if the bacterial DNA was inserted in the correct orientation, if the insert carried a translational initiation signal and if the reading frame on the insert matched that of the *lacI-lacZ* coding sequences of pLG400 or of the *lacZ* coding region of pMC1403. The fragments and vectors used in these constructions are listed in Table 2.

As pRL98 and pRL99 both transform a *cysJ* strain to Cys⁺ (Fig. 4), this suggests that the *cysJ* gene is located on DNA common to the inserts in these two plasmids, namely from *SphI* site 4 to *BglII* site 2 in *λdcysJIHD* DNA. That the *cysJ* gene is present between these sites was confirmed by the ability of pRL100 to transform a *cysJ* strain to Cys⁺ (Fig. 4). The *cysJ* gene is present in the insert of pRL3: the insert of pRL3 therefore has DNA common to pRL100. Since pRL81 can transform a *cysH* strain to Cys⁺, the *cysH* gene must lie to the left of the *HindIII* site 3 in *λdcysJIHD* DNA. pRL5 and pRL7 transform a *cysD* strain to Cys⁺. Thus the *cysD* gene is located between *SmaI* site 4 and the novel joint at the right end of the bacterial DNA carried by *λdcysJIHD*. The failure of pRL6 to transform a *cysD* mutant to Cys⁺ indicates that *BamHI* site 5 lies within the *cysD* gene.

In order to test whether some constructs carried a complete copy of the *cysJ* gene or the *cysD* gene, assays for the enzymes encoded by these genes were done. To further test for cysteine regulation of expression, the assays were done for cells grown on cysteine, which leads to a low or repressed enzyme level, or on djenkolic acid, a poor sulphur source, which leads to a high or derepressed enzyme level. The derepression ratio (derepressed level/repressed level) provides an index of cysteine regulation. For inserts in the fusion vectors pLG400 and pMC1403, *β*-galactosidase assays were done on cells grown under both conditions. Demonstration of cysteine regulation of *β*-galactosidase would enable the direction of transcription from the cysteine regulated promoter on the insert to be deduced.

The derepression ratios for certain plasmids are shown in Table 3. pRL9 has NADPH-cytochrome *c* reductase under cysteine control. Thus the *cysJ* gene and its promoter are located within the insert carried by pRL9 but the degree of cysteine control of *β*-galactosidase is in comparison much lower. Likewise, the cysteine regulation of ATP sulphurylase from pRL5 shows that the *cysD* gene and its promoter are present in the insert. By contrast, the regulation by cysteine of *β*-galactosidase was insignificant when compared to the ATP sulphurylase results. Only for pRL6 is *β*-galactosidase clearly regulated by a cysteine controlled promoter.

DISCUSSION

The size of 9.9 kb for the double stranded region of the heteroduplex molecules with λ dcysJIHD to the right of the attachment site (H) corresponds to the loss of λ DNA on formation of λ dcysJIHD from the λ map coordinates of 27731 bp (*att*) to about 38600 bp. The secondary site lysogen which yielded λ dcysJIHD was a Cys⁺ strain. Thus the prophage in this strain is not located in a *cys* gene. Since λ dcysJIHD transduces to Cys⁺ mutants having mutations in the *cysJ*, *I*, *H* and *D* genes but not the *cysC* gene then the prophage in the secondary site lysogen must be located near the *cysJ* gene end of the cluster but beyond the cluster. Thus the *cys* genes on λ dcysJIHD must be oriented as shown in Fig. 3. The determination of λ dcysJIHD as being a bio-type transducing phage establishes the orientation of the prophage in the secondary site lysogen which yielded λ dcysJIHD (strain RL75) as: *N* gene of λ - *J* gene of λ - *cysJ* - *cysI* - *cysH* - *cysD*.

The λ DNA lost on formation of λ dcysJIHD probably corresponds to the loss of the *N* and *cI* genes. The fact that the phage is defective is consistent with the loss of gene *N*.

The heteroduplex molecules observed with λ dcysJIHD and λ cysJ DNA had no deletion loops corresponding to the expected *nin5* deletion. That the 5804 bp *EcoRI* fragment and the 6527 bp *BamHI* fragment of λ DNA were present in the respective digests of λ dcysJIHD and λ cysJ DNA confirms that the *nin5* deletion (from 40.6 to 43.4 kb of λ DNA, Szybalski & Szybalski, 1979) is not present in each transducing phage DNA.

For λ cysJ DNA, both heteroduplex analysis and restriction enzyme analysis are consistent with the loss of no or very little λ DNA due to the bacterial DNA insertion. If none has been lost then the bacterial DNA may be derived from the two regions of the chromosome which are adjacent to the two ends of the prophage in the secondary site lysogen. Transducing phage of this type have been reported by Britton *et al.* (1983). If this is so then the novel joint between these two regions of bacterial DNA should be located to the left of the position in λ cysJ which is aligned with the *BglII* site 2 in λ dcysJIHD DNA as depicted in Fig. 3. Another possibility is that during the excision event on formation of λ cysJ other recombination and deletion events have occurred such as reported by Jorgensen *et al.* (1978).

From the restriction enzyme analysis, λ cysJ has a length of 52.1 ± 1.3 kb and λ dcysJIHD has a length of 51.8 ± 0.7 kb. Thus they are both close to 107% of wild-type length. Feiss *et al.* (1977) have observed packaging recoveries of 100% for phage genomes which are 105% of wild-type length but 20% for genomes of 109% of wild-type length.

The location by cloning experiments of the *cysJ* and *cysD* genes in λ dcysJIHD DNA (see Fig. 4) confirms the predicted orientation of the gene cluster carried by this phage shown in Fig. 3. From the order of genes of the cluster derived by Jones-Mortimer (1973), the *cysH* gene must lie between the *cysJ* and *cysD* genes, i.e. to the right of the *cysJ* gene, and from cloning experiments reported here (Fig. 4) it lies to the left of *HindIII* site 3 at coordinate 26.90 (kb). Since the *cysD* gene is located to the right of *SmaI* site 4 at coordinate 38.88, there is a large 'silent section' of DNA of at least 11 kb in which no known cysteine genes are located. This is the first evidence for the physical size of this region in either *E. coli* K12 or *S. typhimurium*. Previously the term 'silent section' has been used for the section between the *cysH* and *cysD* genes of *S. typhimurium* which was estimated to be about as long as the remainder of the cluster from recombination frequency data (Demerec *et al.*, 1963).

One of the conditions for β -galactosidase to be expressed on cloning fragments into pLG400 or pMC1403 is that the insert must contain a promoter in the correct orientation. The direction of transcription of the *cysJ* gene is unknown. If it is transcribed from left to right in λ dcysJIHD DNA (as depicted in Fig. 3) then the insert in pRL9 is in the wrong orientation for expression of *lacZ* from this promoter. This could be the reason that a comparatively low level of cysteine control of *lacZ* was observed.

The cysteine control of β -galactosidase demonstrated for pRL6 and the mapping of the orientation of the insert establish that the cysteine regulated promoter on the insert directs transcription from left to right with respect to λ dcysJIHD DNA. Since the insert of pRL7 contains the *cysD* gene (see Fig. 4) and the *BamHI* site 5 is within the gene (see Results) the promoter detected on pRL6 must be that for the *cysD* gene. This establishes the orientation of the *cysD* gene on λ dcysJIHD DNA and from this the orientation on the *E. coli* chromosome is

such that it is transcribed anticlockwise with respect to the linkage map (Bachmann, 1983). For pRL5 the *cysD* gene promoter is present in the correct orientation but no control of *lacZ* expression by cysteine was observed. The complete *cysD* gene is present as judged from the ATP sulphurylase results and it is not clear whether another reading frame under cysteine control would be fused to *lacZ*. The reason for the level of β -galactosidase observed for cells grown in the presence of cystine is unknown.

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