Co-transduction with *serB* of a Pleiotropic Mutation Affecting Colicin E2 Refractivity, Ultraviolet Sensitivity, Recombination Proficiency and Surface Properties of *Escherichia coli* K12

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SUMMARY

A *cetB* locus, determining E2 refractivity at low temperature, has been mapped at minute 89 on the *Escherichia coli* K12 chromosome and is 94 % linked to the *hsp* loci. A second locus, *cetA*, which controls expression of *cetB* in some way, was found to be 70 % co-transducible with and to the right of *thr*. Both *cetB* and *cetC*, another locus which determines E2 refractivity, are 21 % co-transducible with *serB* and the *cetC* locus therefore also probably lies close to *hsp*. Mutations of the *cetC* locus appear to be pleiotropic and the additional characteristics of these mutants, u.v. sensitivity, detergent sensitivity, recombination deficiency, filament formation, and abortive λ growth, are all fully co-transducible with E2-refractivity. In addition, u.v.-resistant revertants of *CetC* mutants simultaneously revert to all wild-type characteristics including E2 sensitivity. The nature of the recombination deficiency of some *CetC* mutants has been examined and both episomal and chromosomal gene transfer to the mutants found to be normal. P1 transduction frequencies with *CetC* mutants are, however, also normal and it is suggested that the mutants may be deficient in some recombination step unique to $Hfr \times F^-$ crosses.

INTRODUCTION

Mutants of *Escherichia coli* which still adsorb colicin E2 but which are, nevertheless, refractory to its effects, particularly at low temperature, are designated *Cet*. These mutants may be divided into two phenotypic groups, those, *CetC*, which are u.v.-sensitive and show other pleiotropic effects and those, *CetB*, in which mutation to E2 refractivity is not pleiotropic. The additional characteristics of *CetC* mutants include reduced recombination proficiency, inhibition of the growth of bacteriophage λ and filament formation. The general properties of these mutants including evidence for altered cell surfaces have been described previously and in the accompanying paper (Holland, 1967, 1968; Holland, Threlfall, Holland, Darby & Samson, 1970). Previous genetic analysis of *Cet* mutants using interrupted mating techniques indicated that the *cetB* locus mapped 1-2 min. counter clockwise to *thr*. In addition it was also previously shown that the u.v.-sensitivity associated with the E2-refractivity of *CetC* mutant ASH 112 also mapped close to *thr* (Holland & Threlfall, 1969).

In this paper we describe further genetic analysis of *CetC* mutants and also present additional evidence for the recombination deficiency of some *CetC* mutants. By P1 transduction *cetB* and *cetC* loci have been mapped very close to the *hsp* loci (host

specificity, see Taylor & Trotter, 1967) at minute 89 on the *E. coli* K12 chromosome. Moreover in all cases tested so far, the various mutant characteristics of CetC strains were always found to be 100 % co-transducible with the E2-refractivity of the mutants.

METHODS

Organisms. CetC mutants ASH 111, 112, 113, 114, 115 and 116 and the CetB mutant ASH 102 have all been described in the preceding paper (Holland *et al.* 1970). ASH 5 is an F⁻ strain (*thi⁻ met⁻ arg⁻ thr⁻ leu⁻ gal⁻ trp⁻ his⁻ str^r tsx^r*); Hfr P10, obtained from Professor Hans Kornberg, transfers *argG* and *str* as early markers in a clockwise direction; strain HfrH was that used previously (Holland & Threlfall, 1969); strain 4 K (*serB⁻ hsp⁻ thr⁻ leu⁻ str^r*) was kindly provided by Dr S. Glover. Strain 4 K is defective in both restriction and modification processes. The F' factor KLF-1 (Low, 1968) which covers the *serB-leu* region of the chromosome was provided by Dr Brooks Low. In the studies described here *E. coli* C600 (*thr⁻ leu⁻ λ⁻ lac⁻ str^s*) was always used as F' donor. The *recB* mutant JC 4457 and its *rec⁺* parent AB 1157 were kindly provided by Dr A. J. Clark. The male specific phage μ was obtained from Professor R. H. Pritchard and the virulent phage λ gv was described previously (Holland, 1967).

The cultural conditions used, the source and assay of colicin E2 (P9) and colicin E3 (CA 38) have all been described previously. Methods for linkage analysis of CetC mutants and plate tests for colicin sensitivity determination have also been described elsewhere (Holland & Threlfall, 1969).

Transduction studies with temperate phage P1. Lysates, which usually contained 10¹⁰–10¹¹ p.f.u./ml., of laboratory stocks of P1 assayed and used as described by Glover (1962). Recipient bacteria, grown in Oxoid tryptone broth (TB) to about 2 × 10⁸ bacteria/ml., were resuspended in 1/5 volume of phage buffer (Glover, 1962) containing 10⁻³ M-CaCl₂ and an equal volume of phage was added (m.o.i. of 5 to 10 P1 particles per cell). Adsorption was completed by incubating 90 min at 25°, and the mixture was centrifuged and the pellet resuspended in 0.07 M-phosphate buffer pH 7.2 containing 0.5 % sodium citrate to prevent re-infection on the plate. Aliquots (0.1 ml.) were diluted where necessary and plated on selective plates also containing 0.5 % sodium citrate.

Selection of E2-refractory transductants. The P1 bacterial adsorption mixture described above was incubated for a further 90 min. in nutrient broth (Oxoid no. 2) at 37° before plating in order to allow expression of E2 refractivity in the transductants. Aliquots of the mixture were then plated on nutrient broth (NB) agar plates plus 5 × 10³ units of colicin E2 and the plates incubated 24 h. at 25°. Refractory colonies were then replica-plated to plates containing E2 and to plates containing E3 (5 × 10³ units in total) and incubated at both 25° and 37° to confirm the E2 refractivity phenotype. Scoring for auxotrophic markers amongst refractory transductants was completed by replica plating from pattern grids of the original *cetB* or *cetC* colonies. In reciprocal crosses *serB* transductants were replica plated directly to colicin lawns on NB plates to determine the proportion of the unselected *cet* marker. For final confirmation of the presence of the *cet* allele, transductants were picked, purified and tested for E2 and E3 sensitivity in cross-streak tests against each colicin at both 37° and 25°, as described previously (Hill & Holland, 1967).

Scoring for *hsp⁺*. Cell suspensions of test strains were spotted, in duplicate, on NB

agar plates; the dried spots were half overlaid with a loopful of phage λ gv (10^4 p.f.u./ml) previously grown in the *hsp*⁻ host 4K. After 14 h. at 37°, *hsp*⁻ mutants showed a clear zone of lysis whilst *hsp*⁺ strains showed confluent growth.

Scoring for λ 'resistance'. Test strains were spotted on NB agar as before and half overlaid with λ gv (10^6 pfu/ml.) previously grown on a wild-type host. After 14 h. at 37°, wild-type strains showed an area of lysis whilst resistant mutants like ASH 112 showed confluent growth.

Scoring for u.v.-sensitive transductants. The u.v. source was a low pressure mercury lamp (Hanovia Ltd) with an incident dose rate at room temperature of 6.5 ergs/mm.²/sec. at 46 cm. Transductants to be tested were grown to late exponential phase in NB medium, diluted to about 2×10^7 bacteria/ml. and streaked in duplicate on tryptone broth plates. A stepwise irradiation of successive portions of the inoculated streaks was then carried out; different sections of the streaks received, 60, 40, 20, 15, 10, 5 and 0 sec. of u.v. The plates were incubated in the dark for 14 h. at 37°; sensitive strains were usually inhibited by doses of 5 to 15 sec. whilst wild-type strains were resistant to 40 sec. u.v.

Scoring for detergent sensitivity. Transductants to be tested were grown to late exponential phase (10^8 cells/ml.) in NB medium and streaked in duplicate on each of five NB agar plates containing 2.5, 1.0, 0.5, 0.1 and 0 % sodium deoxycholate (DOC) respectively. Wild-type *cet*⁺ strains were resistant to 1 % or higher whilst *cetC* mutants were completely inhibited by 0.5 % DOC.

Isolation of u.v.-resistant revertants. The procedure used was adapted from that described by Ganesan & Smith (1968). Cultures of *CetC* mutants were grown to stationary phase in TB medium, diluted to 10^7 bacteria/ml. and 0.1 ml. spread on TB agar plates; the plates were exposed to a u.v. dose of 130 ergs/mm.² and incubated in the dark at 37° for 14 h. The plates containing approximately 100 colonies were replica-plated to duplicate TB plates and immediately exposed to a u.v. dose of 520 ergs. After incubation at 37°, colonies which appeared u.v.-resistant were picked from the original plate, purified and u.v.-resistance confirmed by the plate test described above. Only revertants obtained from different cultures of the original *CetC* mutants were considered to be of independent origin.

F' transfer. F' and F⁻ strains were grown to 5×10^7 and 5×10^8 bacteria/ml. respectively in M9 minimal medium (Anderson, 1946) supplemented with required amino acids. Equal volumes were then mixed and shaken slowly at 37°. After 15 or 30 min., streptomycin (100 μ g./ml.) was added to kill the male cells and to stop further transfer. The cultures were immediately diluted and plated on appropriate media for the selection of prototrophic heterozygotes. To confirm the F status of heterozygotes, cultures were spotted on NB agar plates, half over-layered with RNA phage μ (about 10^{10} p.f.u./ml. and incubated at 37° for 8 h.; F⁺ strains showed a half-moon zone of inhibited growth whereas F⁻ strains showed confluent growth.

Determination of β -galactosidase synthesis. Induction of β -galactosidase synthesis by isopropyl-thio- β -D-galactoside (IPTG) and assay of the enzyme were carried out as described by Pardee, Jacob & Monod (1959). The enzyme substrate was *O*-nitrophenol- β -D-galactoside (ONPG) and enzyme activities in toluene extracts of cells were calculated as μ moles of *O*-nitrophenol released/min./ml. of cells suspension. (IPTG and ONPG were obtained from the Sigma Chemical Co., St Louis, Missouri, U.S.A.)

RESULTS

A second gene required for expression of the Cet phenotype

Previous reports (Hill & Holland, 1967; Holland & Threlfall, 1969) indicated that only certain K12 strains are capable of giving rise to Cet mutants after 2-amino purine treatment. These same strains have now been found to be transducible for the Cet character whilst other strains are not. In the course of genetic analysis of various CetB mutants it was observed that a gene potentiating the expression of the *cetB* locus segregated with *thr* in genetic crosses. Mapping studies with transducing phage P1 have now shown that this gene, tentatively designated *cetA*, is 70 % linked to *thr* and probably lies to the right of *thr* (Table 1). We have quite arbitrarily designated those strains which can potentiate the expression of E2 refractivity arising from mutations at the *cetB* locus as mutant, i.e. phenotype CetA, whilst other strains are wild-type. Whether this assignment is correct must await clarification of the role of the *cetA* locus in determining E2 refractivity. We have not yet determined whether the *cetA* locus influences the expression of mutations at the *cetC* locus. The Cet status of various wild-type and mutant strains is summarized in Table 2.

Table 1. Mapping of the *cetA* locus by P1-transduction

P1 donor	Recipient	Selected marker	No. tested	Unselected marker %		Possible transductant classes	No. obtained	%
				<i>leu</i> ⁺	<i>cetA</i>			
HfrH (<i>cetA</i>)	4K (<i>cet</i> ⁺)	<i>thr</i> ⁺	60	6.5	70	<i>thr</i> ⁺ <i>leu</i> ⁺ <i>cetA</i>	4	6.7
						<i>thr</i> ⁺ <i>leu</i> ⁺ <i>cet</i> ⁺	0	0
						<i>thr</i> ⁺ <i>leu</i> ⁻ <i>cet</i> ⁺	18	30
						<i>thr</i> ⁺ <i>leu</i> ⁻ <i>cetA</i>	38	63

Probable gene order and linkage *thr*—*cetA*—*leu*
 ← 70 % →
 ← 6.5 % →

The *thr*⁺ transductants were replica plated to minimal agar to determine the proportion of the *leu*⁺ marker inherited. To determine inheritance of *cetA* *thr*⁺, transductants were picked, purified and exponential cultures of each clone crossed with a P1 donor grown on the *cetB* strain ASH 102. The proportion of the original *thr*⁺ transductants able to receive and express the *cetB* marker was taken as a measure of the frequency of *thr*⁺ *cetA* transductants.

Table 2. Cet status of various *Escherichia coli* K12 strains and refractory mutants

Strain	CetA	CetB	CetC
HfrH	—	+	+
ASH 5	—	+	+
4K	+	+	+
ASH 6	—	+	+
ASH 10	—	+	+
ASH 102	—	—	+
ASH 111	—	+	—
ASH 112	—	+	—
ASH 113	—	+	—
ASH 114	—	+	—
ASH 115	—	+	—
ASH 116	—	+	—

Wild-types are denoted + and mutant phenotypes as —.

Linkage analysis of *CetC* mutants

Previous analysis of 21 *CetB* mutants derived from the parental F^- strain ASH 10 (Holland & Threlfall, 1969) demonstrated that E2 refractivity in all mutants mapped to the left of *thr* and *leu* and was closely linked to this region. As described in Table 3, linkage analysis of several *CetC* mutants derived from ASH 10, in crosses with an HfrH male, showed that E2 refractivity in all these mutants was also linked to *leu* and probably to the left of this locus.

Table 3. Linkage analysis of *CetC* mutants

CetC recipient	Recombinants			E 2-sensitive (<i>cet</i> ⁺) (%)		
	Selected marker	Input male (%)	No. tested			
ASH 112	<i>leu</i> ⁺ <i>str</i> ^r	0.4	72	73		
	<i>ton</i> ^r <i>str</i> ^r	0.12	24	59		
	<i>lac</i> ⁺ <i>str</i> ^r	0.09	96	32		
ASH 113	<i>leu</i> ⁺ <i>str</i> ^r	0.005	46	68		
	<i>ton</i> ^r <i>str</i> ^r	—	—	—		
	<i>lac</i> ⁺ <i>str</i> ^r	0.002	21	30		
ASH 115	<i>leu</i> ⁺ <i>str</i> ^r	2.7	112	78		
	<i>ton</i> ^r <i>str</i> ^r	1.6	25	51		
	<i>lac</i> ⁺ <i>str</i> ^r	0.8	165	34		
ASH 116	<i>leu</i> ⁺ <i>str</i> ^r	3.0	90	70		
	<i>ton</i> ^r <i>str</i> ^r	1.3	17	53		
	<i>lac</i> ⁺ <i>str</i> ^r	0.8	28	40		
				Proportion of unselected marker (%)		
				<i>ton</i> ^r	<i>leu</i> ⁺	<i>cet</i> ⁺
ASH 102	<i>lac</i> ⁺ <i>str</i> ^r	—	103	85	75	59
ASH 112	<i>lac</i> ⁺ <i>str</i> ^r	—	202	90	81	26
ASH 113	<i>lac</i> ⁺ <i>str</i> ^r	—	83	79	64	53
ASH 115	<i>lac</i> ⁺ <i>str</i> ^r	—	84	—	76	44

An HfrH strain (*cet*⁺ *ton*^r λ^- *str*^s) was crossed with various *CetC* derivatives of ASH 10. Exponential cultures (10^8 cells/ml.) of each strain were mixed and incubated 90 min. at 37°; selection was then made for appropriate recombinant classes, and subsequently the proportion of unselected markers including E2-sensitivity (*cet*⁺) amongst the recombinants was determined by replica plating.

Analysis of *CetB* and *CetC* mutants by P1 transduction

For transduction mapping of *CetB* and *CetC* mutations, *Escherichia coli* strain 4K was used as recipient. This strain has convenient markers to the left of *leu* in the order *hsp*⁻ *serB*⁻ *thr*⁻ *leu*⁻ (Glover & Colson, 1969; S. W. Glover, personal communication). Before strain 4K could be used, however, it was first necessary to introduce the *cetA* allele into this strain. This was achieved by crossing strain 4K with a P1 lysate of HfrH (*thr*⁺ *cetA*) and selecting a *thr*⁺ transductant which also carried the *cetA* allele. The new derivative of strain 4K obtained in this way was designated ASH 6.

Bacteriophage P1 was grown on strain ASH 102 (*CetB*) and the lysate used to infect cultures of ASH 6. E2-refractory transductants were selected directly by plating infected cultures on E2 lawns incubated at 25°. After confirming the E2 refractivity of

the transductants by replica plating to plates containing colicin E2 or E3 at both 25° and 40°, the inheritance of the unselected marker *serB*⁺ was determined. The results obtained (Table 4) showed *cetB* and *serB* to be 21 % co-transducible. When the same P1 donor was used, but with *serB*⁺ as the selected marker, *cetB* was found to be 22 % linked to *serB*. Furthermore, when P1 grown on the *CetC* mutant ASH 112 was used, 21 % of ASH 6 *serB*⁺ transductants also inherited the *cetC* locus (Table 4). Since previous studies (Holland, & Threlfall, 1969) indicated that both *cetB* and *cetC* loci were 1 to 2 minutes to the left of *thr*, it appeared probable that the *cet* loci were also to the left of *serB* and close to *hsp*. To confirm the relative order of the *cet* and *serB* loci, three factor crosses were carried out with primary selection for either *serB*⁺ or E2 refractivity and using the *hsp* locus as outside marker. This experiment has so far only been carried out with P1 grown on the *CetB* mutant ASH 102. The increased λ resistance of the *CetC* mutant ASH 112 made scoring for inheritance of the phenotypically similar *hsp*⁺ marker impossible. The results obtained (Table 5) showed an anticipated 19 to 20 % linkage of *serB*⁺ and *cetB*⁺ and also showed *cetB* to be 93 to 94 % linked to

Table 4. *Co-transduction of cetB and cetC with serB⁺*

	P1 donor	Recipient	Selected marker	Trans- ductants per 10 ⁶ P1	No. scored	Unselected marker		
						<i>thr</i> ⁺	<i>leu</i> ⁺	<i>serB</i> ⁺
Expt. 1	HfrH (<i>cet</i> ⁺)	4K	<i>serB</i> ⁺	9.8	200	44	0.5	—
			<i>thr</i> ⁺	6.8	200	—	6.5	47
			<i>leu</i> ⁺	1.8	41	6	—	0
Expt. 2	ASH 102 (<i>cetB</i>)	ASH 6	<i>serB</i> ⁺	1.7	150	100	22	—
			<i>cetB</i> ⁺	1.8	150	21	100	—
			<i>serB</i> ⁺	4.4	435	100	—	21

Transduction procedures and analysis of unselected markers as described in Methods and in Table 2. ASH 6 is a *thr*⁺ *cetA* transductant of strain 4K obtained from a cross between P1 HfrH and 4K (*thr*[−] *cet*⁺).

Table 5. *Analysis of cetB linkage to hsp⁺ and serB⁺ by P1 transduction*

P1 donor	Recipient	Selected marker	Trans- ductants per 10 ⁶ P1	No. scored	Unselected marker (%)		
					<i>serB</i> ⁺	<i>cetB</i>	<i>hsp</i> ⁺
ASH 102	ASH 6	<i>serB</i> ⁺	3.2	250	100	21	18.5
		<i>cetB</i>	2.1	100	—	100	93

Linkage and possible gene order *hsp*—*cetB*—*serB*
 ← 93 → ← 21 →
 ← 18.5 →

Procedures as described in Tables 2 and 3.

the *hsp* loci. The *hsp*⁺ *serB*⁺ linkage was, however, only 17 to 19 %, and these results clearly established the gene order as *cetB serB thr leu* with *cetB* and *hsp* extremely closely linked. Examination of the minority classes of transductants obtained in the crosses shown in Table 6 unfortunately still leaves the relative order of *cetB* and *hsp* ambiguous. Selection for *serB*⁺, with scoring for the unselected markers *hsp*⁺ and *cetB*,

Table 6. Analysis of three-factor crosses involving *hsp*, *serB* and *cetB*

P _I donor	Recipient	Selected marker	Trans-ductants per 10 ⁶ P _I	No. scored	Unselected marker (%)			Possible transductant classes			No. obtained	%	
					<i>ser</i> B ⁺	<i>cet</i> B	<i>hsp</i> ⁺	<i>hsp</i> ⁺	<i>hsp</i> ⁺	<i>hsp</i> ⁺			
Expt. 1.	ASH 102	<i>ser</i> B ⁺	0.7	166	—	18.5	17.5	0	<i>ser</i> B ⁺	<i>hsp</i> [−]	<i>cet</i> ⁺	134	80.7
									<i>ser</i> B ⁺	<i>hsp</i> [−]	<i>cet</i> B	3	1.8
									<i>ser</i> B ⁺	<i>hsp</i> ⁺	<i>cet</i> B	28	16.8
									<i>ser</i> B ⁺	<i>hsp</i> ⁺	<i>cet</i> ⁺	1	0.6
Indicated gene order													
<i>hsp</i> ⁺ — <i>cet</i> B— <i>ser</i> B ⁺													
← 18.5 →													
← 17.5 →													
Expt. 2	ASH 6	<i>cet</i> B	3.4	160	19	—	94	0	<i>ser</i> B ⁺	<i>hsp</i> ⁺	<i>cet</i> B	30	19
									<i>ser</i> B ⁺	<i>hsp</i> [−]	<i>cet</i> B	1	0.7
									<i>ser</i> B [−]	<i>hsp</i> [−]	<i>cet</i> B	8	5.0
									<i>ser</i> B [−]	<i>hsp</i> ⁺	<i>cet</i> B	121	76.5
Indicated gene order													
<i>cet</i> B— <i>hsp</i> ⁺ — <i>ser</i> B ⁺													
← 94 → ← 19 →													

Procedures as described in Tables 2 and 3.

Table 7. Co-transduction with *cetC* of additional phenotypic characters of *CetC* mutants

Expt.	P ₁ donor	Recipient	Selected marker	Trans-ductants per 10 ⁶ P ₁	No. scored	No. of transductants tested and scored as:					λ
						E2 refractory (<i>cetC</i>)	<i>doc</i> ^a	<i>uv</i> ^a	<i>rec</i> ^{-(a)}	<i>fil</i> ^{-(c)}	
Expt. 1	ASH 112	ASH 6	<i>cetC</i>	0.3	19	19	19	19	—	—	—
	ASH 113	ASH 6	<i>cetC</i>	0.4	38	38	12/12 ^(b)	12/12	38	—	—
Expt. 2	ASH 112	ASH 6	<i>serB</i> ⁺	4.4	435	103	103	103	—	—	—
Expt. 3	ASH 112	ASH 5	<i>cetC</i>	3.2	14	14	14	14	1/1 ^(b)	—	14
	ASH 113	ASH 5	<i>cetC</i>	1.9	4	4	4	4	4	—	—
	ASH 114	ASH 5	<i>cetC</i>	2.1	14	14	14	14	1/1	—	14
	ASH 111	ASH 5	<i>cetC</i>	2.3	14	14	14	14	1/1	14 ^(c)	—

Transduction procedures as described in Tables 2 and 3. Scoring for unselected markers as described in Methods.

^(a) To determine recombination deficiencies purified clones were crossed with HfrH (Expt. 1) and the deficiency of *leu*⁺ *str*^r recombinants determined, or with Hfr P10 (Expt. 3) when deficiency of *arg*⁺ *str*^r recombinants was measured.^(b) This indicates that 12 out of 12, or 1 out of 1, transductants tested displayed the mutant phenotype.^(c) On initial isolation all showed filament formation, after nutrient broth subculture only three were still found to be filament-forming.

indicated a gene order *hsp cetB serB*. When selection was made for *cetB* the frequencies of unselected markers indicated an order *cetB hsp serB*. Further crosses, with examination of considerably more transductants will be required before the final order can be established.

Genetic analysis of other phenotypic characters of CetC mutants

In a previous study (Holland & Threlfall, 1969) the mutation to u.v. sensitivity in the CetC mutant ASH 112 was also mapped close to the *cet* locus. This indicated that u.v.-sensitivity may be a pleiotropic effect of the CetC mutation in this strain and not the expression of an independent gene.

To establish whether u.v. sensitivity in this and in other CetC mutants was co-transducible with E2 refractivity, bacteriophage P1 was first grown on ASH 112 and ASH 113, and the lysates obtained were used to infect the recipient strain ASH 6. E2-refractive transductants were selected directly and, after purification and confirmation of the Cet phenotype, were scored for u.v. sensitivity. Since the donor strains ASH 112 and 113 are detergent-sensitive and recombination deficient, these characters were also scored amongst CetC transductants. The results presented in Table 7 show that all three unselected markers, u.v. and detergent sensitivity and recombination deficiency, for both donor strains were 100 % co-transducible with E2 refractivity. With ASH 112 as donor, primary selection was also made for *serB*⁺ and of 103 *serB*⁺, *cetC* transductants obtained all were also u.v.- and detergent-sensitive.

Other phenotypes associated with CetC mutants include filament formation (ASH 111) and increased resistance to bacteriophage λ (ASH 112 and ASH 114). The results for the transduction of these markers, shown in Table 7, demonstrate that they also are fully co-transducible with *cetC*. In the recipient strain ASH 5 used in these studies, the *fil* character was, however, unstable and upon subsequent subculture of the majority of the transductants, filament formation was lost although the other mutant characteristics of the ASH 111 donor were retained. The transduction studies with the male CetC mutants ASH 111 and 114 also revealed that these strains carry a *rec* marker. This is more clearly illustrated in Table 10.

The results described above give a strong indication that the E2 refractivity and u.v. and detergent sensitivity of CetC mutants derives from a mutational event at a single gene, the *cetC* locus. These studies also indicate that the recombination deficiency, filament formation and increased λ resistance of the mutants are determined by one or more loci closely linked to, if not identical with, the *cetC* locus (Fig. 1).

Isolation of u.v.-resistant revertants of ASH 112 and ASH 113

To further clarify the relationship between E2 refractivity and the other phenotypic changes characteristic of CetC mutants, u.v.-resistant revertants of ASH 112 and ASH 113 were selected by the method described by Ganesan & Smith (1968). Six revertants isolated from each of the two strains have now been studied. All were also sensitive to colicin E2 and resistant to the detergent DOC. Moreover, when two revertants from each group were tested for growth characteristics, all showed normal growth rates in liquid culture, in contrast to the reduced growth rates of the CetC parental strains. Similarly, as shown in Table 8, the revertants tested for recombination proficiency all proved to be much more fertile than the parental mutant strains. One revertant,

Fig. 1. Summary of P I transduction analysis of *Cet* mutants.

ASH 13-R2 although not as recombination-proficient as wild-type *cet*⁺ strains, nevertheless, yielded ten times more recombinants than the original strain ASH 113.

A P1 transduction analysis of revertant strain ASH 112-R1 was carried out to determine whether the *cetC* allele was still present but suppressed in some way. Table 9 shows the results of crosses between P1 donors, ASH 112 and ASH 112-R1 and the recipient strain ASH 6. Selection was made for *serB*⁺ and the transductants scored for inheritance of the *cetC* marker. With the mutant strain as donor, 20 % of *serB*⁺ transductants were also E2 refractory. In contrast, with the revertant strain as P1 donor none of the 320 *serB*⁺ transductants tested were refractory to E2. The site of reversion to u.v. resistance (and to E2 sensitivity) in ASH 112-R1 must therefore reside within the *cetC* locus or be very closely linked to it. Thus the properties of the u.v.-resistant revertants so far studied further support the hypothesis that u.v. sensitivity and the other characteristics of *CetC* mutants, including E2 refractivity, are the pleiotropic expression of a single gene.

Table 8. *Recombination proficiency of some CetC mutants and their u.v.-resistant revertants*

Donor	Recipient	Recombinants % input male <i>lac</i> ⁺ <i>str</i> ^r	Recombination deficiency
HfrH	ASH 10 (<i>cet</i> ⁺)	1.5	—
HfrH	ASH 112 (<i>cetC</i>)	0.18	8
HfrH	ASH 113 (<i>cetC</i>)	0.025	60
HfrH	ASH 112-R1	1.7	—
HfrH	ASH 113-R1	1.0	1.7
HfrH	ASH 113-R2	0.25	6.8

Mating conditions as described in Table 1.

Recombination deficiency index is calculated from ratio of recombinants formed in control cross to that obtained with *CetC* recipient strain. ASH 112-R1 is a u.v.-resistant revertant of ASH 112, ASH 113-R1 and 113-R2 are u.v.-resistant revertants of ASH 113.

The method of isolation of revertants is described in Methods.

Table 9. *Linkage of serB⁺ and cetC loci in ASH 112 and ASH 112-R1*

P1 donor	Recipient	Trans- ductants per 10 ⁶ P1	Selected marker	No. scored	E2-refractive transductants (%)
ASH 112 (<i>cetC</i>)	ASH 6	4.5	<i>serB</i> ⁺	430	21
ASH 112-R1	ASH 6	5	<i>serB</i> ⁺	320	0

Transduction procedure as described in Tables 2 and 3.

ASH 112-R1 is a u.v.-resistant revertant of ASH 112 (see text).

The nature of the recombination deficiency of CetC mutants

Recombination frequencies obtained in crosses with Hfr males

Characteristic feature of some *CetC* mutants is the formation of reduced numbers of recombinants in crosses with male strains (Table 10). With Hfr P10, a male strain transferring the *cet*⁺ locus late, recombination deficiencies of 100 and 400 were obtained with various *CetC* mutants. Similar results have been obtained previously with *RecB* mutants (Emmerson, 1968; Willetts & Clark, 1969). Unlike *Rec* mutants,

however, ASH 113 still showed a large recombination deficiency in crosses with a male strain (HfrH) which transfers the *cet*⁺ locus early. Nevertheless, as shown below, *cetC* is recessive to *cet*⁺. These results therefore indicate that expression of the dominant *cet*⁺ allele in the F⁻ recipient is delayed and that this delay is sufficient to allow an abortive recombination event to occur.

Table 10. *Recombination deficiency of CetC strains and their derivatives in crosses with Hfr strains*

	Donor	Recipient	Recombinants as % of input male			Recombination deficiency index
			<i>leu</i> ⁺ <i>str</i> ^r	<i>ton</i> ⁺ <i>str</i> ^r	<i>lac</i> ⁺ <i>str</i> ^r	
Expt. 1	HfrH	ASH 10 (<i>cet</i> ⁺)	2.2	1.9	0.7	—
	HfrH	ASH 112 (<i>cetC</i>)	0.33	0.17	0.09	7
	HfrH	ASH 113 (<i>cetC</i>)	0.005	—	0.002	440
	HfrH	ASH 102 (<i>cetB</i>)	2.3	1.7	0.6	—
			<i>arg</i> ⁺ <i>str</i> ^r	<i>his</i> ⁺ <i>str</i> ^r	<i>leu</i> ⁺ <i>str</i> ^r	
Expt. 2	HfrP 10	ASH 5 (<i>cet</i> ⁺)	1.3	0.5	0.04	—
	HfrP 10	ASH 5-112 (<i>cetC</i>)	0.007	0.0014	0.0005	180
	HfrP 10	ASH 5-113 (<i>cetC</i>)	0.003	0.0007	0.0001	430
	HfrP 10	ASH 5-111 (<i>cetC</i>)	0.012	0.002	0.0007	110
	HfrP 10	ASH 5-114 (<i>cetC</i>)	0.006	0.004	0.0003	220

Mating conditions as described in Table 1.

The recombination deficiency index was determined from the frequency of *leu*⁺ (HfrH) or *arg*⁺ (HfrP10) recombinants obtained in each cross relative to that obtained with the parental strains ASH 10 or ASH 5. The *cetC* derivatives of ASH 5 were obtained by crossing P1-*cetC* donors ASH 112, 113, 111 and 114 with ASH 5 and selection made for E2 refractory transductants as described in Methods.

Table 11. *Episome transfer to CetC mutants*

Donor episome	Recipient	Frequency of transfer (% input male)
F' <i>lac</i> ⁺	ASH 10 (<i>cet</i> ⁺)	1.6
	ASH 102 (<i>cetB</i>)	2.2
	ASH 112 (<i>cetC</i>)	2.1
	ASH 113 (<i>cetC</i>)	1.0
	ASH 115 (<i>cetC</i>)	1.9
	ASH 116 (<i>cetC</i>)	0.06
KLF-1	ASH 102 (<i>cetB</i>)	3.7
F' <i>serB</i> ⁺ - <i>leu</i> ⁺	ASH 112 (<i>cetC</i>)	1.8
	ASH 115 (<i>cetC</i>)	2.4
	ASH 116 (<i>cetC</i>)	0.1

Cultures of F' and F⁻ strains grown in M9 supplemented medium to 5×10^7 and 5×10^8 cells/ml. respectively; cultures mixed and shaken slowly for 15 min. before plating to determine frequency of episome transfer. Episome donor strain in each case was c 600 (*thr*⁻ *leu*⁻ *lac*⁻ *str*^R).

Efficiency of gene transfer to CetC mutants

Since recombination deficiency in F⁻ recipient strains could result from incorrect pairing with the male donor, it was necessary to determine whether gene transfer to *CetC* mutants was normal or otherwise. The efficiency of episome transfer was first tested and, as shown in Table 11, the transfer of F' *lac*⁺ or episome KLF-1 to most *CetC* mutants, including the recombination deficient strains ASH 112 and 113, was

almost normal. Conjugal pairing with male strains appears therefore to be unimpaired for the majority of *CetC* mutants. Although not previously found to be deficient in recombination with *HfrH*, one strain, ASH 116, did show a 25-fold reduction in transfer of either episome. The reason for this is not clear, and the properties of this strain will require further study.

A direct measurement of chromosomal gene transfer from *Hfr* males to *CetC* mutants was also carried out by measurement of β -galactosidase synthesis in merozygotes. *CetC* mutants ASH 112 and 113 are both *lac*⁻ (*z*⁻) and make insignificant levels

Fig. 2. β -Galactosidase formation in merozygotes. (a) Male (*HfrH*) and female strains ASH 112, 113 and ASH 10 were grown in NB media at 37° to 2×10^8 and 4×10^8 cells/ml. respectively. At time 0 β -galactosidase synthesis was induced by the addition of IPTG (0.25 mM) and samples removed at intervals and enzyme content determined as described in Methods. In the case of strain *HfrH* (*lac*⁺), induction was carried out either in the absence or presence of streptomycin (100 μ g./ml.) added at -10 min. (b) Cultures of male and female strains at 4×10^8 and 2×10^7 cells/ml. respectively were mixed and shaken slowly at 37° for 30 min. Streptomycin (SM) was then added and the culture blended vigorously for 60 sec. to terminate mating. The cultures were then centrifuged and resuspended in 1/20 volume. Inducer IPTG was then added and samples removed at intervals to determine enzyme content as before.

of β -galactosidase in the presence of lactose. As shown in Fig. 2, however, the capacity for β -galactosidase synthesis by these mutants, after 30 min. mating with an *HfrH* (*lac*⁺) male, was identical to that of *cet*⁺ females in similar crosses. Thus the transfer of chromosomal *lac*⁺ genes by a male donor to both ASH 112 and 113 is quite normal. The results shown in Fig. 2 also make it unlikely that transferred DNA is rapidly

destroyed in the recombination deficient mutants since β -galactosidase synthesis appears to continue at the normal rate for at least 90 min. These strains thus appear to be recombination-deficient as a result of a defect in the molecular mechanism of recombination, including chromosomal pairing, or through the failure of potential recombinants to grow, i.e. as a result of a lethal zygotis of some kind.

Formation of transductants by *CetC* mutants

Luria & Hertman (1967) showed that a *RecA* mutant formed transductants at the same low frequency ($< 10^{-3}$) as for recombinant formation after conjugation. *RecB* strains, in contrast, appear to yield transductants at frequencies as high as 5 to 25 % of wild-type *rec*⁺ strains (Emmerson & Howard-Flanders, 1967; A. J. Clark, personal communication). The formation of *leu*⁺, *met*⁺ and *thy*⁺ transductants in the *CetC* mutants ASH 112 and 113 occurs with almost the same frequency as that obtained with *cet*⁺ strains (Table 12). The *RecB* mutant JC 4457 included as a control in this experiment gave an anticipated four- to seven-fold reduction in transduction formation. This result, together with the physiological evidence presented in the preceding paper (Holland *et al.* 1970), clearly indicates that the basis of the recombination deficiency of *CetC* mutants in crosses with Hfr males is quite different from that found in either *RecA* and *RecB* mutants.

Table 12. Transduction frequencies obtained with *CetC* mutants

P I donor	Recipient	Transductants per 10 ⁶ P I particles			
		<i>thy</i> ⁺	<i>leu</i> ⁺	<i>his</i> ⁺	<i>arg</i> ⁺
HfrH	ASH 102 (<i>cetB</i>)	6.2	1.8	—	—
HfrH	ASH 112 (<i>cetC</i>)	4.9	1.2	—	—
HfrH	ASH 113 (<i>cetC</i>)	6.0	1.0	—	—
HfrH	AB 1157 (<i>rec</i> ⁺)	—	2.1	5.5	4.4
HfrH	JC 4457 (<i>recB</i> ⁻)	—	0.5	0.8	0.7

Transduction procedure as described in Tables 1 and 2.

Properties of strains heterozygous for *CetB* or *CetC*

The F' factor KLF 1 (Low, 1968) includes the region containing *cetB* and *cetC* and probably also the *cetA* locus, since the factor spans the region from the origin of HfrH to a point beyond *leu*. F' KLF-1 was originally derived from HfrH, a strain which, using the criteria indicated above, we have designated *cetA*, and is it assumed that the KLF 1 factor also carries the mutant allele *cetA* rather than the wild-type *cet*⁺ allele.

Transfer of the KLF 1 factor to either ASH 102 or ASH 112 was readily achieved by selection for *leu*⁺ *str*^r exconjugants in crosses between these strains and strain C600 carrying the F' factor. The presence of the episome in the heterozygotes was confirmed by tests for sensitivity to the male specific phage μ and by secondary transfer of the F' factor to other recipients. When tested for colicin sensitivity, both *cetB* and *cetC* heterozygotes proved to be sensitive to E2. Thus the chromosomally located *cetB* or *cetC* alleles are both recessive to the *cet*⁺ allele on the F' factor.

DISCUSSION

Cet mutants are readily divisible into two major phenotypic groups; those which are u.v.-sensitive and those which are not. A *cetB* locus has now been mapped close to the *hsp* loci at minute 89 on the *Escherichia coli* chromosome. Like *cetB*, *cetC* is 21 % linked to *serB* and therefore this locus also probably lies close to *hsp*. It is not yet clear whether *cetB* and *cetC* loci are in fact distinct cistrons, but complementation studies are in progress which should clarify this point. In view of the extremely close linkage between *cetC* and *hsp*, a locus controlling chemical modification and degradation of DNA (Arber, 1968; Meselson & Yuan, 1968) the response of *hsp*⁻ mutants to E2 has also been tested. Such mutants are not refractory to E2 (Holland, 1967) and, moreover, E2-induced DNA breakdown proceeds normally (unpublished results). Thus the *cet* and *hsp* loci are quite distinct although the frequency of co-transduction indicates, according to the formula of Wu (1966), that these genes are only 0.04 minutes or 3 to 4 genes apart.

The results presented here further support in at least three ways the hypothesis (Holland, 1967) that mutations at the *cetC* locus are pleiotropic: (i) all the mutant phenotypes exhibited by CetC mutants, in particular u.v.-sensitivity, DOC sensitivity and recombination deficiency, are fully co-transducible with E2 refractivity; (ii) Ultraviolet resistant revertants simultaneously revert to all the *cet*⁺ wild-type characteristics; (iii) 2-aminopurine, the mutagen used to isolate the mutants, has not so far been reported to induce multisite mutations.

An unusual property of CetC mutants, not incompatible with pleiotropic gene action, is the way in which various characteristics including E2 refractivity, u.v.-sensitivity and filament formation, can be temporarily and independently lost whilst other characteristics are retained. Such changes are not due to reversion and, indeed, despite the reduced growth rate of some CetC strains the mutants are quite stable. Most, if not all, of the mutant phenotypes displayed by CetC mutants are therefore conditional in an as yet unknown way, upon some aspect of cellular growth and physiology. It is also clear that expression of the *CetB* and probably *CetC*, mutants is dependent upon the activity of the *cetA* locus and clarification of the function of this gene may be required before the properties of the mutants are properly understood. In addition, the Cet mutants have now been shown to carry the amber or an ochre suppressor (unpublished results), and this also may profoundly affect the mutant phenotype. Efforts are now being made to eliminate this suppressor from the mutants and also to construct partial diploids, homozygous for *cetC*, in the hopes that this will lead to some stabilization of the properties of the mutants.

Although the reason for the recombination deficiency of CetC mutants is still not clear, the genetical evidence presented here and the biochemical evidence presented previously, which indicates that the majority of mutants show no major alteration in nuclease functions (Holland, 1967; Holland *et al.* 1970), clearly distinguishes these strains from previously studied Rec mutants. Despite the obvious cell surface alterations of CetC mutants, the formation of conjugal pairs and the process of gene transfer appear to be quite normal. Whilst some non-specific effect, leading for instance, to killing of the CetC recipient as a result of conjugation, cannot yet be ruled out, it appears more likely that chromosomal pairing or an enzymic step in the recombination process itself is defective in the mutants. In contrast to Rec⁻ mutants

including a *RecB* strain tested in this study, *CetC* recipient strains give practically normal transduction frequencies with phage P1. This may indicate a deficiency in the mutants unique to some step in $HfrH \times F^-$ crosses, e.g. chromosomal pairing, which has no effect upon recombination processes involving small transducing fragments. It should be noted, however, that in the studies reported here, P1 multiplicities of infection of 10 were normally used. Under these conditions multiple infection is likely to occur and complementation by normal phage genomes of some defective host recombination function may take place. Since temperate P1 phage was used, normal transductants could form and survive in the lysogenic cell by this mechanism. This possibility will be explored in future studies. To establish whether or not the mutants are truly defective in a specific DNA recombination step, attempts will also be made to measure β -galactosidase synthesis in merozygotes under conditions in which enzyme could only be formed following recombination of male and female DNA.

As discussed in the preceding paper, the pleiotropic effects observed in *CetC* mutants could either arise from polar mutations or from mutations affecting an important functional site in the cell membrane. Since the varied characteristics of *CetC* mutants have as a common basis alteration of surface properties and disturbance of DNA metabolism, an attractive candidate for an altered membrane component is that normally concerned with replication and segregation of DNA (Ryter, 1968; Sueoka & Quinn, 1968). Comparison of the constitution of membranes from wild-type and mutant strains is now being carried out to test this hypothesis.

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