Location of the Aspartase Gene (*aspA*) on the Linkage Map of *Escherichia coli* K12

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SUMMARY

The aspartase (L-aspartate ammonia-lyase, EC. 4.3.1.1) structural gene, *aspA*, was mapped by two-factor and three-factor transductional crosses using phage P1. Cotransduction frequencies between *aspA* and other markers were: *ampA* (69%); *frdA* (48 to 67%); *mel* (35%); *purA* (17%); *fdp* (1.6%). The sequence of markers within the corresponding segment (91 to 95 min) of the *Escherichia coli* linkage map was shown to be *mel-aspA-ampA-frdA-purA-fdp*.

INTRODUCTION

Wild-type strains of *Escherichia coli* K12 fail to grow on glutamate as sole carbon and energy source because the transport of this amino acid is severely repressed. Permeability to glutamate is determined by three genes: *gltS*, the glutamate permease structural gene, and two regulatory genes, *gltC* and *gltR*. The *gltC* gene, or operator locus, is very close to *gltS* but the *gltR* gene, which specifies the repressor of permease synthesis, is not closely linked (Marcus & Halpern, 1969*a*). Mutations in the *gltR* or *gltC* genes permit good growth on glutamate. Studies with *gltC* mutants (constitutive mutants) have shown that aspartase and aspartate aminotransferase are essential for growth on glutamate because mutants (*aspA*) lacking aspartase and one mutant (*aspC*) lacking the aminotransferase are defective in this respect (Marcus & Halpern, 1969*b*). Furthermore, the synthesis of aspartase is increased with glutamate as substrate (Halpern & Umbarger, 1960; Vender, Jayaraman & Rickenberg, 1965). The evidence indicates that aspartase, together with the aminotransferase, fumarase and malate dehydrogenase, functions in a 'deamination cycle' which effects the conversion of glutamate to 2-oxoglutarate and regenerates the amino-acceptor, oxaloacetate (Fig. 1).

Aspartase is also considered to be involved in the formation of succinate during anaerobic growth on glucose. This was discovered by Courtright & Henning (1970) during studies with malate dehydrogenase mutants (*mdh*) of *E. coli*. Such mutants are unable to convert phosphoenolpyruvate plus CO₂ to succinate by the route involving phosphoenolpyruvate carboxylase, malate dehydrogenase, fumarase and fumarate reductase. However, the alternative route involving aspartate aminotransferase and aspartase provides an effective by-pass of the *mdh* block (Fig. 1). Furthermore, during anaerobic growth malate dehydrogenase is repressed whereas aspartase is derepressed, indicating that even in the wild type a major fraction of the succinate is probably synthesized anaerobically *via* aspartate.

Conjugation studies with two aspartase mutants have placed the *aspA* gene in the 92 to 94 min region of the chromosome (Marcus & Halpern, 1969*c*) where the fumarate reductase gene (*frdA*) has also been located (Spencer & Guest, 1973). Since both enzymes function in the anaerobic synthesis of succinate it was of interest to determine whether these two

Fig. 1. Metabolic pathways for the metabolism of glutamate and the anaerobic synthesis of succinate in *E. coli* illustrating the role of aspartase (Marcus & Halpern, 1969*b*; Courtright & Henning, 1970). The enzymes, and the corresponding structural genes with their approximate locations (in min) on the *E. coli* linkage map (Bachmann, Low & Taylor, 1976), are: glutamate permease (*gltS*, 81); aspartate aminotransferase (*aspC*, 20); aspartase (*aspA*, 92); fumarate reductase (*frdA*, 93); phosphoenolpyruvate carboxylase (*ppc*, 88) and malate dehydrogenase (*mdh*, 69). Reactions concerned with glutamate metabolism are denoted by closed-headed arrows, reactions leading to succinate under anaerobic conditions by open-headed arrows, and reactions common to both functions by the hybrid arrows.

metabolically-related genes are sufficiently close to consider the possibility of them constituting an operon. In the present work the position of the *aspA* gene relative to *frdA* and several other neighbouring genes was mapped by transduction using phage P1.

METHODS

Organisms. The sources and characteristics of the primary set of strains of *Escherichia coli* K12 are listed in Table 1. Strains CS8, possessing the *gltC8* mutation which causes constitutive synthesis of the glutamate permease, and CS8ASP/23, an *aspA* mutant of CS8, were kindly provided by Dr Y. S. Halpern. A variety of derived strains were constructed by transduction using phage P1; they are all presumed to be P1 lysogens and their characteristics and derivations are summarized in Table 2. The Glt⁺ phenotype refers to the ability to grow with glutamate as sole carbon and energy source. Strains of *E. coli* normally have a Glt⁻ phenotype. However, many of the constructed strains are Glt⁺ because they possess the *gltC8* mutation and so express the glutamate permease gene (*gltS*) constitutively. In selecting Glt⁺ derivatives of the basic strains, CS8(*gltC8*) was used as the donor; relatively high frequencies of transduction [10 to 45 per 10⁶ P1 plaque-forming units (p.f.u.)] were observed, except with ymel as recipient when the frequency was 0.08 per 10⁶ P1 p.f.u. This ymel strain, a λ-sensitive derivative of the classical strain, was an auxotroph, incapable of growth on glucose minimal medium without nutritional supplementation. Consequently, the prototrophic ymel/G strain is probably the product of a double event, for example, transduction to prototrophy plus mutation to Glt⁺ or transduction to Glt⁺(*gltC8*) plus reversion to prototrophy. For this reason there is some doubt about the *gltC8* designation of ymel/G and its derivatives (Table 2). The *fdp* mutation of strain Q11 precluded direct

Table 1. *Strains of Escherichia coli* K12

Strain	Characteristics	Source
CS8	Hfr, <i>met</i> , <i>gluC8</i> (λ)	Y. S. Halpern (Marcus & Halpern, 1967)
CS8ASP/23	Hfr, <i>aspA</i> , <i>met</i> , <i>gluC8</i> (λ)	Y. S. Halpern (Marcus & Halpern, 1969b)
R4frd	Hfr, <i>frdA1</i> , <i>met</i>	Spencer & Guest (1973)
WGASfrd	F ⁻ , <i>frdA11</i> , <i>gal</i> , <i>trpA9761</i> , <i>trpR</i>	P. R. Lambden
wpur	F ⁻ , <i>purA</i> , <i>trpR</i>	W. T. Drabble (originally RM1003, a mutant of W3110)
G11a1	Hfr, <i>ilv</i> , <i>metB</i> , <i>ampA</i> (λ)	K. G. Eriksson-Grennberg (Eriksson-Grennberg, 1968)
KG20	F ⁺ , <i>pro</i> , <i>his</i> , <i>argH</i> , <i>thi</i> , <i>purA</i> , <i>ampA</i> , <i>str</i>	K. G. Eriksson-Grennberg (Eriksson-Grennberg, 1968)
Q11	Hfr, <i>fdp</i>	K. G. Eriksson-Grennberg (Fraenkel & Horecker, 1965)
ymel	F ⁺ , <i>mel-1</i> , <i>supE57</i> , <i>supF58</i>	W. J. Brammar (Bachmann, 1972)
W3110	F ⁻ , <i>trpR</i>	W. J. Brammar (Bachmann, 1972)

Table 2. *Strains of Escherichia coli* K12 derived in the present work

Derived strains were prepared by phage P1-mediated transduction and are assumed to be P1 lysogens. The designation 'G' refers to the presence of the *gluC8* (constitutive) mutation of cs8 and 'a' denotes ampicillin-resistance conferred by the *ampA* mutation of G11a1.

Strain	Characteristics	Derivation
R4frd/G	<i>frdA1</i> , <i>met</i> , <i>gluC8</i>	Glt ⁺ transductant from cs8 into R4frd
R4frd/a	<i>frdA1</i> , <i>met</i> , <i>ampA</i>	Amp ^R transductant from G11a1 into R4frd
R4frd/ag	<i>frdA1</i> , <i>met</i> , <i>ampA</i> , <i>gluC8</i>	Glt ⁺ transductant from cs8 into R4frd/a
R4/G	<i>met</i> , <i>gluC8</i>	Frd ⁺ (AspA ⁺) transductant from CS8ASP/23 into R4frd/G
R4/Gasp	<i>met</i> , <i>gluC8</i> , <i>aspA</i>	Frd ⁺ (AspA ⁻) transductant from CS8ASP/23 into R4frd/G
WGASfrd/G	<i>frdA11</i> , <i>gal</i> , <i>trpA9761</i> , <i>trpR</i> , <i>gluC8</i>	Glt ⁺ transductant from cs8 into WGASfrd
wpur/G	<i>purA</i> , <i>trpR</i> , <i>gluC8</i>	Glt ⁺ transductant from cs8 into wpur
w/G	<i>trpR</i> , <i>gluC8</i>	Pur ⁺ (Asp ⁺) transductant from CS8ASP/23 into wpur/G
w/Gasp	<i>trpR</i> , <i>gluC8</i> , <i>aspA</i>	Pur ⁺ (AspA ⁻) transductant from CS8ASP/23 into wpur/G
w/Gfdp	<i>trpR</i> , <i>gluC8</i> , <i>fdp</i>	Pur ⁺ (Fdp ⁻) transductant from Q11 into wpur/G
KG20/G	<i>pro</i> , <i>his</i> , <i>argH</i> , <i>thi</i> , <i>purA</i> , <i>ampA</i> , <i>str</i> , <i>gluC8</i>	Glt ⁺ transductant from cs8 into KG20
ymel/G	<i>met</i> , <i>gluC8</i>	Glt ⁺ prototrophic transductant from cs8 into ymel
ymel/Ga	<i>met</i> , <i>gluC8</i> , <i>ampA</i>	Amp ^R transductant from G11a1 into ymel/G
Y/G	<i>gluC8</i>	Mel ⁺ (AspA ⁺) transductant from CS8ASP/23 into ymel/G
Y/gasp	<i>gluC8</i> , <i>aspA</i>	Mel ⁺ (AspA ⁻) transductant from CS8ASP/23 into ymel/G

selection of a Glt⁺ derivative by transduction with the cs8 donor, so this mutation was introduced into the Glt⁺ background of wpur/G by selecting Pur⁺ transductants. Of 116 Pur⁺ transductants w/Gfdp was one of seven which were unable to grow on glycerol minimal medium (Fdp⁻). This corresponds to a *purA*-*fdp* cotransduction frequency of 6% which is in fair agreement with the value of 14% reported by Eriksson-Grennberg (1968). Most of the remaining derivatives were products of the crosses described below. Note that since the *aspA* mutation generates a Glt⁻ phenotype in *gluC8* (constitutive) strains, the Glt⁻ phenotype can be synonymous with AspA⁻.

The genetic symbols are those used in the current *E. coli* linkage map (Bachmann, Low & Taylor, 1976) and the phenotypic abbreviations are directly related to them.

Media. The basal minimal medium contained (per litre): KH₂PO₄, 5.44 g; K₂HPO₄, 10.49 g; (NH₄)₂SO₄, 2 g; MgSO₄·7H₂O, 0.05 g; MnSO₄·4H₂O, 5 mg; FeSO₄·7H₂O, 0.125 mg; CaCl₂, 0.5 mg. Substrates were added to give the following final concentrations (mM): D-glucose, 10; mono-sodium L-glutamate, 30; glycerol, 40; α -D-melibiose, 5; sodium fumarate, 40; potassium succinate, 40. The glycerol plus fumarate medium also contained vitamin-free Casamino acids (Difco; 0.5 g l⁻¹). Media were supplemented with amino acids, purines, pyrimidines and vitamins according to the particular requirements of

the bacteria to be grown or selected. All media used in the primary selection of transductants were also supplemented with Difco nutrient broth (2 ml l^{-1}). Anaerobic incubation was carried out in an atmosphere of H_2 plus CO_2 (95:5, v/v).

Bacterial stocks were maintained on L agar (Lennox, 1955) and L broth was the complex medium used for routine subculture. Media were solidified with Bacto agar (15 g l^{-1}) as required. All cultures were incubated at 37°C .

Growth experiments. Cultures were shaken in 250 ml Erlenmeyer flasks fitted with optically matched side-arms. Each flask contained 10 ml of glutamate minimal medium plus L-methionine ($20 \mu\text{g ml}^{-1}$) inoculated with 0.2 ml of a washed suspension of organisms to give an initial extinction at 610 nm of 0.08 (approximately $0.04 \text{ mg dry wt bacteria ml}^{-1}$). The inocula were prepared from cultures of organisms grown for 20 h on methionine-supplemented succinate minimal medium, washed and resuspended in saline.

Assay of aspartase. Cultures were grown for 20 h in stationary 1 l Erlenmeyer flasks filled to the neck with a complex medium containing (per litre): peptone, 4 g; yeast extract, 4 g; K_2HPO_4 , 6 g; glucose, 10 g (added after autoclaving). The pH was 6.8 and the inoculum was 1 ml of an overnight L broth culture of the corresponding strain. Cultures were harvested at 4°C , washed twice in cold potassium phosphate buffer (0.01 M ; pH 7.8) and resuspended at a concentration of $0.25 \text{ g wet wt ml}^{-1}$. These suspensions were disrupted at 0°C by two 2 min treatments with an ultrasonic disintegrator (MSE, 100 W). After centrifuging at 15000 g for 20 min, the supernatant fluids were assayed for aspartase. Protein concentrations were measured by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Aspartase (L-aspartate ammonia-lyase, EC. 4.3.1.1) was assayed at 20°C with a reaction mixture containing: potassium L-aspartate, $100 \mu\text{mol}$; $\text{Co}(\text{NO}_3)_2$, $0.3 \mu\text{mol}$; potassium phosphate buffer (pH 7.2), $100 \mu\text{mol}$; and water plus extract in a final volume of 1.0 ml. Ammonia production was measured at different times by adding 3.4 ml KOH (0.4 M) and 0.05 ml Nessler Reagent (BDH). After 10 min, extinctions were read at 492 nm and the values were compared with a standard curve prepared using $\text{Na}(\text{NH}_4)\text{HPO}_4$. Aspartase activities are quoted in terms of $\mu\text{mol NH}_3$ released ($\text{mg protein}^{-1} \text{ h}^{-1}$) at 20°C .

Genetic methods. Transductions were performed with the temperate phage Pr_1 . Lysates of donor strains were prepared by confluent lysis using soft agar overlays (2.5 ml Difco nutrient broth plus Bacto agar, 6 g l^{-1}) seeded with 5×10^8 bacteria and 10^6 phages before pouring on plates of L agar containing Bacto agar (10 g l^{-1}) and CaCl_2 (2.5 mM). Incubation was for 5 to 6 h after which L broth (3.3 ml per plate) was added, and the plates were kept at 2°C for 18 h. The lysate was pipetted from the surface of the plates, treated with CHCl_3 and titrated for plaque-forming phages using *Shigella dysenteriae* as indicator. For transduction, CaCl_2 (0.05 ml ; 0.05 M) was added to 1×10^9 stationary-phase recipient bacteria in 0.2 ml L broth and pre-incubated for 30 min at room temperature. Lysate containing 2×10^9 phages in 1 ml L broth was added and the phages were adsorbed at 37°C for 25 min. Unadsorbed phages were removed by sedimenting the bacteria and appropriate dilutions of the transduced culture were plated on selective media. Controls containing L broth but no phages were included to test for reversion of the recipient, and the sterility of the lysates was checked. Reversion was occasionally detected in experiments with derived *aspA* strains, but this was never greater than 1% of the transduction frequency in the experiments quoted.

Transductants were selected on appropriate media with major substrates: glucose (Pur^+); glutamate (Glt^+ and AspA^+); glycerol (Fdp^+); melibiose (Mel^+); glycerol plus fumarate incubated anaerobically (Frd^+). Amp^R transductants were selected on L agar containing

Fig. 2. Growth on glutamate minimal medium of several strains of *E. coli* K12. Cultures (10 ml) were shaken at 37 °C in 250 ml Erlenmeyer flasks fitted with optically matched side-arms and their extinctions at 610 nm were measured at regular intervals over a period of 24 h (see Methods).

DL-ampicillin ($10 \mu\text{g ml}^{-1}$) after the transduced cultures had been resuspended in L broth plus sodium citrate (5 mM) and incubated for 4 h to permit expression of the Amp^R phenotype. Transductants were counted after 1 to 5 days, depending on the selective medium, and streaked on unenriched selective medium for scoring the inheritance of unselected markers by replica-plating. With Amp^R recipients, transductants were purified by streaking to single colonies on the selective medium before scoring non-selective markers.

RESULTS

Characteristics of the aspartase mutant and derived strains

A characteristic of mutants (*aspA*) of *E. coli* K12 which lack aspartase is their failure to grow with glutamate as sole carbon and energy source, even when the glutamate transport system is derepressed (Marcus & Halpern, 1969b). In fact the Glt⁻ nutritional phenotype of *aspA* mutants can only be detected in backgrounds which are constitutive or derepressed for glutamate uptake. Thus CS8(*gltC8*) was able to grow on glutamate but its derivative CS8ASP/23 (*gltC8*, *aspA*) had a Glt⁻ phenotype. However, in preliminary transduction studies designed to test for linkage between the aspartase (*aspA*) and fumarate reductase (*frdA*) loci, no Glt⁺ transductants were obtained with CS8ASP/23 as recipient. This was observed not only with donor strains such as R4frd and W3110, which have a Glt⁻ phenotype although they possess a wild-type *aspA* gene, but also with the parental strain CS8, which is Glt⁺ and AspA⁺. It was therefore concluded that this culture of CS8ASP/23 contained at least two transductionally-unlinked mutations preventing growth on glutamate. Similar results were obtained when transductants were selected on aspartate minimal medium. Consequently, CS8ASP/23 could only be used as a donor strain in studies on the location of the *aspA* gene. This necessitated the construction of Glt⁺ (*gltC*, constitutive) derivatives of all strains carrying markers in the relevant region of the chromosome (91 to 95 min) for use as recipients. These constructions were made by transduction with strain CS8(*gltC8*) as donor (see

Table 3. *Aspartase activities of primary and derived strains*

Aspartase was assayed in ultrasonic extracts of organisms grown anaerobically in complex medium as detailed in Methods. The specific activities are expressed as $\mu\text{mol NH}_3$ released $(\text{mg protein})^{-1}\text{h}^{-1}$ at 20°C.

Strain	Phenotype	Aspartase (specific activity)
CS8	Glt ⁺	0.72
CS8ASP/23	Glt ⁻	< 0.01
R4frd	Glt ⁻	1.01
R4frd/G	Glt ⁺	0.84
R4/G	Glt ⁺	0.41
R4/Gasp	Glt ⁻	< 0.01

Methods and Table 2). In subsequent transductions with CS8ASP/23 as donor, selections for Frd⁺, Pur⁺, Fdp⁺ and Mel⁺ all led to the segregation of Glt⁺ and Glt⁻ derivatives (Table 2; crosses 1 to 5, Table 4). Clearly, CS8ASP/23 contains a mutation which is cotransducible with these markers and specifies a Glt⁻ phenotype.

The nature of this mutation was examined in detail with several transductants. The growth on glutamate liquid medium of one family of Glt⁺ and Glt⁻ strains derived from R4frd is illustrated in Fig. 2. The parental strain R4frd was unable to use glutamate but introduction of the *gltC8* mutation from CS8 to yield R4frd/G permitted growth on glutamate. Of the Frd⁺ transductants obtained from R4frd/G with CS8ASP/23, R4/G (Glt⁺) grew as well as R4frd/G and R4/Gasp (Glt⁻) resembled R4frd and CS8ASP/23 in being unable to grow on glutamate. Similar results were obtained with comparable strains: w/G (Glt⁺) plus w/Gasp (Glt⁻); and ymel/G (Glt⁺), Y/G (Glt⁺) plus Y/Gasp (Glt⁻). In no case did the Glt⁺ (*gltC8*) derivatives grow as well as CS8; the reason for this is not clear.

Ultrasonic extracts of anaerobically-grown cultures of R4frd and its derivatives were assayed for aspartase and the specific activities were compared with those observed for similar extracts of CS8 and CS8ASP/23 (Table 3). Like CS8, strains R4frd, R4frd/G and R4/G all contained aspartase whereas no activity was detected in either CS8ASP/23 or R4/Gasp (the Frd⁺Glt⁻ transductant obtained by crossing CS8ASP/23 with R4frd/G). Clearly CS8ASP/23 lacks aspartase; its *aspA* mutation is closely linked to the *frdA* gene and transduced with it to generate the Glt⁻ phenotype of R4/Gasp. Thus in R4/Gasp the Glt⁻ phenotype is synonymous with an AspA⁻ phenotype. It is probable that the same is true for the other Glt⁻ (AspA⁻) derivatives such as w/Gasp and Y/Gasp since they were constructed by analogous methods involving markers linked to the *frdA* gene. It is interesting that the Glt⁻ (AspA⁻) derivatives reverted fairly readily on glutamate minimal medium. In contrast, CS8ASP/23 was extremely stable and no Glt⁺ revertants were ever detected. This is a further indication that the Glt⁻ phenotype of CS8ASP/23 is due to more than one mutation.

The Glt⁻ phenotype of R4frd, which has considerable aspartase activity (Table 3), is undoubtedly a consequence of the severe repression of glutamate uptake normally found in *E. coli* K12.

Genetic mapping by transduction

Two-factor crosses. The results of tests for P1-mediated cotransduction between pairs of markers in the 91 to 95 min region of the *E. coli* linkage map are presented in Table 4. With CS8ASP/23 as donor the *aspA* mutation, which generated a Glt⁻ (AspA⁻) phenotype, was cotransducible with *frdA*, *purA*, *fdp* and *mel* (crosses 1 to 5, Table 4). Reciprocal crosses involving *frdA* and *mel* donors with a derivative *aspA* strain, w/Gasp, confirmed the cotrans-

Table 4. Transductional linkage relationships in the *mel* to *purA* region

Cross	Donor	Recipient	Selection	Transductants per 10 ⁶ P1	Number scored	Unselected donor marker	Cotransduction frequency (%)
1	CS8ASP/23	R4frd/G	Frd ⁺	9	225	AspA ⁻	65.7
2	CS8ASP/23	WGAsfrd/G	Frd ⁺	20	127	AspA ⁻	67.7
3	CS8ASP/23	wpur/G	PurA ⁺	3	176	AspA ⁻	17.0
4	CS8ASP/23	w/Gfdp	Fdp ⁺	2	186	AspA ⁻	1.6
5	CS8ASP/23	Ymel/G	Mel ⁺	17	167	AspA ⁻	35.9
6	G11a1	R4/Gasp	Amp ^R	1	196	AspA ⁺	74.0
			AspA ⁺	12	213	Amp ^R	69.5
7	G11a1	w/Gasp	Amp ^R	3	210	AspA ⁺	68.1
			AspA ⁺	8	241	Amp ^R	65.1
8	WGAsfrd	w/Gasp	AspA ⁺	9	244	Frd ⁻	48.4
9	Ymel	w/Gasp	AspA ⁺	11	220	Mel ⁻	34.1
10	G11a1	Ymel/G	Amp ^R	9	170	Mel ⁺	31.2
			Mel ⁺	45	287	Amp ^R	25.7
11	Ymel	WGAsfrd	Frd ⁺	81	188	Mel ⁻	23.9
12	Ymel	wpur	PurA ⁺	40	336	Mel ⁻	4.8

Table 5. Three-factor crosses involving *aspA*, *ampA*, *purA*, *frdA* and *mel* markers

Cross	Bacterial strains and relevant phenotype		Selection	Transductants per 10 ⁶ P1	Number scored	Distribution of unselected markers		Gene order indicated
	Donor	Recipient				Type	No.	
13	CS8ASP/23 (AspA ⁻)	KG20/G (Amp ^R PurA ⁻)	PurA ⁺	19	264	Amp ^S AspA ⁻	42	<i>aspA-ampA-purA</i>
						Amp ^S AspA ⁺	18	
						Amp ^R AspA ⁻	8	
						Amp ^R AspA ⁺	196	
14	CS8ASP/23 (AspA ⁻)	Ymel/Ga (Amp ^R Mel ⁻)	Mel ⁺	24	121	Amp ^S AspA ⁻	16	<i>mel-aspA-ampA</i>
						Amp ^S AspA ⁺	1	
						Amp ^R AspA ⁻	7	
						Amp ^R AspA ⁺	97	
15	CS8ASP/23 (AspA ⁻)	R4frd/aG (Amp ^R Frd ⁻)	Frd ⁺	23	226	Amp ^S AspA ⁻	174	<i>aspA-ampA-frdA</i>
						Amp ^S AspA ⁺	49	
						Amp ^R AspA ⁻	0	
						Amp ^R AspA ⁺	3	

ducibility of *frdA* with *aspA* and *mel* with *aspA* (crosses 8 and 9, Table 4). Comparable reciprocal tests for linkage between *purA* and *aspA* were not performed because of the unexplained observation that the PurA⁻ class of transductants is never recovered from crosses involving a *purA* mutant as donor (Spencer & Guest, 1973). Likewise, the reciprocal cross involving an *fdp* mutant donor is precluded because selection for AspA⁺ transductants on glutamate medium demands the presence of an active fructose 1,6-bisphosphatase for gluconeogenesis.

The linkage between *aspA* and *ampA* was studied with two derivative *aspA* mutants as recipients, R4/Gasp and w/Gasp (crosses 6 and 7, Table 4). In each case Amp^R and AspA⁺ transductants were selected and the four cotransduction frequencies were comparable.

During the studies involving the *aspA* marker, cotransduction frequencies were also determined for *mel* with *ampA*, *frdA* and *purA* (crosses 10, 11 and 12, Table 4).

The results of the two-factor crosses are averaged and summarized in the upper part of the linkage map shown in Fig. 3. Other relevant cotransduction frequencies determined previously by Eriksson-Grennberg (1968), Spencer & Guest (1973) and Siegel & Ivers (1975), are included in the lower part of the map. Clearly *aspA* is close to the *ampA* and *frdA*

Fig. 3. Linkage map of *E. coli* from approximately 91 to 95 min showing the positions of markers in this region and their cotransduction frequencies. The data above the map summarize the average cotransduction frequencies derived from two-factor crosses. The head of each arrow points to the marker selected and the cotransduction frequency corresponding to each selection is that nearest to the marker selected. The data below the map were obtained in earlier studies: *a*, Spencer & Guest (1973); *b*, Eriksson-Grennberg (1968); *c*, Siegel & Ivers (1975).

markers and marginally closer to *ampA* than *frdA*. A comparison of the cotransduction frequencies for the pairs of markers *aspA*–*purA* and *aspA*–*fdp* (17 % and 1.6 %) with *frdA*–*purA* and *frdA*–*fdp* (39 % and 6 %) also indicates *aspA*–*ampA*–*frdA*–*purA*–*fdp* as the most probable order, because the relative orientations of the last four markers is already established. Linkage studies involving the *mel* marker place it to the left of all the other markers shown in Fig. 3, and indicate that *aspA* is located between *mel* and *ampA*.

Three-factor crosses. The position of *aspA* relative to *ampA* was determined by the three-factor crosses shown in Table 5. A P1 lysate of CS8ASP/23, the *aspA* donor, was crossed with three *ampA* mutants: KG20/G, Ymel/Ga and R4frd/aG, constructed so that *purA* (cross 13), *mel* (cross 14) and *frdA* (cross 15) could serve as the outside markers to be selected. The selected transductants were carefully purified by streaking to single colonies before scoring the distribution of the unselected *ampA* and *aspA* markers.

In cross 13 the Amp^sAspA[–] class of Pur⁺ transductant was the least frequent and, if this is presumed to derive from a quadruple cross-over, the order *aspA*–*ampA*–*purA* is confirmed. The *purA*–*aspA* linkage (18.9 %) is in reasonable agreement with the two-factor data obtained in cross 3 (17 %) and the *purA*–*ampA* linkage (22.7 %) is slightly lower than values reported previously (24 to 42 %, Fig. 3).

In cross 14 the least frequent class of Mel⁺ transductant was Amp^sAspA⁺ and, by the same reasoning, this supports the order *mel*–*aspA*–*ampA*. The *mel*–*aspA* and *mel*–*ampA* cotransduction frequencies (19 % and 14 %) were low compared with the corresponding frequencies obtained in the two-factor crosses (35.9 % in cross 5 and 25.7 % in cross 10, respectively).

Cross 15 suffered from the disadvantage that the selected outside marker, *frdA*, is extremely close to the *ampA* marker. Nevertheless, of the four possible classes of Frd⁺ trans-

ductant, the absence of only those with an $\text{Amp}^R\text{AspA}^-$ phenotype confirms the order *aspA-ampA-frdA*. The cotransduction frequencies for *frdA-aspA* and *frdA-ampA* (77 % and 98.7 %) are in reasonable agreement with the corresponding two-factor data (67 % in crosses 1 and 2, and 96 % in Fig. 3, respectively).

DISCUSSION

The results demonstrate that *Escherichia coli* strain CS8ASP/23 lacks aspartase and that the corresponding *aspA* mutation is located in the 92 to 94 min region of the *E. coli* linkage map. This confirms the observations of Marcus & Halpern (1969*b, c*). The cotransduction frequencies for pairs of markers in this region are summarized in Fig. 3 and the gene order was further established by the results of the three-factor crosses. Clearly, the *aspA* gene lies between *mel* and *ampA*. The fact that *aspA* is at best only 67 % cotransducible with *frdA* indicates a separation of at least 0.25 min or 10^4 base pairs (Bachmann, Low & Taylor, 1976). This distance is sufficient for approximately 10 'average' genes, suggesting that the metabolically-related *aspA* and *frdA* genes are unlikely to be components of the same operon. Such a possibility is further precluded by the placing of a functionally-unrelated gene, *ampA*, between the *aspA* and *frdA* genes. However the individual genes could still form part of a regulon controlled by common factors.

The non-random distribution of functionally-related genes at 50 or 25 min intervals in the *E. coli* linkage map has been noted and interpreted in terms of two sequential duplications of an ancestral genome during the evolution of the present chromosome (Zipkas & Riley, 1975). In this context, the *aspB* (68 min), *mdh* (69 min) and *aspC* (20 min) genes are 25 to 27 min from the *aspA* and *frdA* genes and possibly more significant is the 23 min interval between the *frdA* and *sdh* genes, since both specify membrane-bound flavoproteins which catalyse the same metabolic reaction.

The existence of at least two transductionally-unlinked mutations preventing growth of CS8ASP/23 on glutamate was apparent from its failure to yield Glt^+ transductants even with its parent, CS8, as donor. Strain CS8ASP/23 may have suffered further mutation since its isolation because the aminotransferase mutant (CS8TA, *aspC*) and two aspartase mutants CS8ASP/23 and CS8ASP^{TS}, (*aspA*) were originally considered to be due to single point mutations because they reverted spontaneously to a Glt^+ phenotype at high frequencies (Marcus & Halpern, 1969*b*). This behaviour was observed with the *aspA* strains derived during the present work, for example R4/Gasp, W/Gasp and Y/Gasp, but the culture of CS8ASP/23 proved extremely stable. However, any complexities in the genotype of the original *aspA* mutants would probably not have been detected during the early conjugation studies of Marcus & Halpern 1969*b, c*). Nevertheless, the present work indicates that the *aspA* mutation at approximately 92.5 min is responsible for the aspartase defect and the Glt^- phenotype. The nature and location of the other mutation(s) in CS8ASP/23 were not investigated.

Neighbouring genes, not examined in the present work, include *dnaB*, *uvrA* and *mop*. Since *mel* is cotransducible with *dnaB* and *uvrA* (16 % and 30 % respectively; R. Schmitt, personal communication) as well as with *aspA* (35 %), *aspA* should be cotransducible with *uvrA* and possibly *dnaB*, the gene order being *dnaB-uvrA-mel-aspA* not *uvrA-dnaB-mel-aspA* as shown by Bachmann, Low & Taylor (1976). The location of *mop* relative to the genes studied cannot be predicted with certainty. Cotransduction frequencies for *mel-mop* (22 % and 32 %) and *mop-purA* (18 % and 20 %) indicate that *mop* is further from *mel* and slightly closer to *purA* than *aspA* (Takano & Kakefuda, 1972; Sternberg, 1973; Coppo *et al.*, 1973).

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