Journal of General Microbiology (1977), **99**, 157–169
Printed in Great Britain

Location of the Gene Specifying Hexose Phosphate Transport (*uhp*) on the Chromosome of *Escherichia coli*

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(*Received* 28 *July* 1976)

SUMMARY

The *uhp* gene, which specifies the uptake of hexose phosphates, and several other genes in the vicinity of minute 81 on the *E. coli* linkage map have been located by phage-mediated transductions. The order found is *mtl-gpsA-pyrE-gltC-uhp-tna-dnaA*. Alleles specifying the Uhp^- and Uhp^+ characters were separated from that specifying constitutivity of hexose phosphate uptake (Uhp^c). Although cotransduction frequencies between *gltC* and *uhp* as high as 90 %, and between *uhp* and *tna* as high as 80 %, were observed, these frequencies were unusually strongly dependent on which marker was selected. This may be due to the proximity of the *uhp* region to the point of origin of chromosome replication.

INTRODUCTION

Hexose phosphates can be used as such by *Escherichia coli* (Fraenkel, Falcoz-Kelly & Horecker, 1964; Pogell *et al.*, 1966) and are taken up by the organisms via an inducible, energy-dependent transport system. This system shows several interesting properties, the most unusual being its induction by external, but not internal, glucose 6-phosphate (Heppel, 1969; Dietz & Heppel, 1971*b*; Winkler, 1970, 1971). The uptake of hexose phosphates is coupled to a proton gradient generated either by electron transport or by ATP hydrolysis (Winkler, 1973; Essenberg & Kornberg, 1975).

These findings were established with the aid of mutants that were either devoid of the uptake system for hexose phosphates (*uhp*: Kornberg & Smith, 1969) or constitutive for expression of this system (*uhp*^{c}; Ferenci, Kornberg & Smith, 1971). The mutants were found to map in the region of minute 81 on the *E. coli* linkage map as revised by Bachmann, Low & Taylor (1976) and were about 50 % cotransducible with *pyrE*. Kadner & Winkler (1973) confirmed and extended these observations and showed that the *uhp* locus was on the opposite side of *pyrE* from the *cysE* and *mtl* markers. Kadner (1973) subsequently mapped many alleles in the *uhp* area and divided the region into a structural and regulatory portion based on whether or not reversions from *uhp* mutants gave rise to constitutive mutants. He confirmed the observations of Ferenci *et al.* (1971) that constitutive lesions and negative lesions were closely linked, but was not able to determine their orientation directly.

To locate the *uhp* marker more closely, we studied the cotransduction frequencies of *uhp* and *uhp*^{c} with several other genes in the region of minute 81 which were not tested by Kadner & Winkler (1973) and which might be more closely linked to *uhp* than is *pyrE*. The results are reported in this paper.

Table 1. *Escherichia coli strains used in this study*

Strain	Genotype	Source and reference
K10	HfrC	Laboratory stock
AT2243	HfrC *metB pyrE*	A. L. Taylor
236	*mtlA leu thi*	E. C. C. Lin (Solomon & Lin, 1972)
239	*mtlD mtlC*^{c} *leu thi*	E. C. C. Lin (Solomon & Lin, 1972)
CY115	*gpsA metE trpE xyl tsx str*	J. E. Cronan, Jr (Cronan & Bell, 1974)
CS7	*met gltC*^{c}	Y. S. Halpern (Marcus & Halpern, 1969)
AB2147	*ilv argH metB his tna gal lac Y* or *Z malA ara xyl str tsx thi* λ^{R} λ^{-}	B. Bachmann (Pittard & Walker, 1967)
E177	*thi thr leu thyA dra dnaA*^{ts} *lac Y str tonA* λ^{-} *supE*	B. Bachmann (Wechsler & Gross, 1971)
RE21	HfrC *metB pyrE uhp-40 tna*	AT2243 by EMS
RE30	HfrC *metB pyrE uhp-41*	AT2243 by EMS
RE37	HfrC *metB pyrE uhp-2*	AT2243, 2-deoxyglucose 6-phosphate resistant
AT2243-11^{c}	HfrC *metB pyrE uhp*^{c}	AT2243 (Ferenci *et al.*, 1971)
RE21U	HfrC *metB uhp-21 tna*	RE21 × K10, PyrE^{+} transductant
RE30U	HfrC *metB uhp-30*	RE30 × K10, PyrE^{+} transductant
RE37U	HfrC *metB uhp-2*	RE37 × K10, PyrE^{+} transductant
AT2243-11^{c}U	HfrC *metB uhp*^{c}	AT2243-11^{c} × K10, PyrE^{+} transductant

METHODS

Media. LB is the tryptone/yeast extract medium described by Luria & Burrows (1957). Medium 56 (Monod, Cohen-Bazire & Cohn, 1951) containing thiamin.HCl at 1 μg ml^{-1} was diluted 1:2 before use and was supplemented with carbon sources at 10 mM (except glucose 6-phosphate which was used at 5 mM). Amino acids, purines and pyrimidines were added, as required, to final concentrations of 100 μg ml^{-1}. When used in plates, these media were solidified with 2 % (w/v) agar (Difco).

Bacteria and bacteriophage. The strains of *E. coli*, their genotypes and their sources are listed in Table 1. The symbols used for genetical markers are those listed by Bachmann *et al.* (1976).

Overnight cultures for experiments were started from single colonies. Bacteriophage P1*vir* was obtained from Dr E. J. Murgola, Section of Molecular Biology, University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025, U.S.A. It was propagated in chosen strains of *E. coli* and the phage titres were measured by the procedure given by Miller (1972), except that the bacteriophage were grown in organisms plated on LB medium containing 5 mM-$CaCl_2$, using a top agar described by Goldberg, Bender & Streicher (1974). Bacteriophage P1*cml clr100* was obtained from Dr J. L. Rosner, Laboratory of Molecular Biology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014, U.S.A. Lysates and lysogens were prepared as described by Rosner (1972), except that the 40 °C step in the thermal induction was not used.

Mutagenesis and selection of strains. Two main methods of mutagenesis were employed. Ethyl methanesulphonate (EMS) mutagenesis was performed on strain AT2243 as described by Lin, Lerner & Jorgensen (1962), whereas the method of Schwartz & Beckwith (1969) was used when nitrous acid was the mutagen. In most cases, a penicillin-enrichment step (Gorini & Kaufman, 1960) was interposed before selecting mutants. Mutants were identified either by replica plating, as those showing reduced growth on plates containing 1 mM-glucose 6-phosphate as sole carbon source compared with growth on glucose, or by searching for microcolonies on plates containing a mixture of 1 mM-glucose 6-phosphate and 0·25 mM-glucose.

We also attempted to isolate *uhp* mutants by plating cultures of *E. coli* on media containing glycerol and 2-deoxyglucose 6-phosphate (Dietz & Heppel, 1971*a*). Although one such mutant was obtained by this technique (RE37; Table 1), the method was less satisfactory than those described above and was abandoned.

Putative mutants revealed by any of these techniques were picked, tested again on the media on which they had been isolated and then purified by repeated isolation of single colonies on LB plates.

Bacteriophage P1 *transductions.* Transductions using either P1*vir* or P1*cml clr100* were performed as described by Miller (1972). For a given lysate, preliminary transductions were done using different dilutions of bacteriophage to determine the ratio of phage to cells which gave most transductants. Further transductions were performed using a single mixture of bacteria and bacteriophage at this ratio, which was plated on as many selective plates as necessary to obtain a sufficient number of recombinants. Sodium citrate was used to prevent re-infection in all cases. Control plates of bacteria alone, and bacteriophage alone, were used for all crosses. After recombinants appeared on the selective plates (usually 2 days at 37 °C), they were picked with sterile toothpicks and transferred to fresh selective plates which were grown overnight at 37 °C: these organisms were then replicated to test for inheritance of the unselected markers. Exceptions to this general procedure are noted in the legends to the Tables.

Analysis of the results of the crosses. In attempting to analyse the four-factor crosses, we found that there was no general procedure for determining the orientations of markers. We therefore developed a technique which also proved useful in the more common three-factor crosses, and was used for all the crosses reported in this paper. Two types of analysis were done on four- and higher-factor crosses. The first was to compute cotransduction frequencies, both between the selected marker and the unselected markers and also between pairs of unselected markers. From the latter, considered in groups of three, one can construct an order as follows. The lowest frequency of cotransduction will occur between the pair of markers furthest apart. These must therefore be the outside two, with the third between. This procedure is rigorous if the selected marker lies between the outermost markers, but sometimes gives misleading results if the selected marker lies well outside this region. From the cotransduction frequencies one can determine which unselected marker is closest to the selected marker, but not on which side it is.

The second type of analysis resolves this ambiguity and alone can provide an order for a three-factor cross. It is an extension of the concept of linkage and is based on the fact that unselected markers on opposite sides of the selected marker generally have no effect on each other's inheritance; thus if the two unselected markers are A and B, both A^+ and A^- recombinants will show equal ratios of B^+/B^- recombinants, that is $A^+B^+/A^+B^- = A^-B^+/A^-B^-$. If A and B are on the same side of the selected marker, one class of recombinants between A and B will require four crossovers while the other will require only two, so the ratios of B^+/B^- recombinants will not be equal in A^+ and A^- progeny. To test these data for a significant deviation from independent segregation, a χ^2 value was calculated, using Yates' (1934) correction, for the 2×2 contingency table with rows for A^+ and A^- progeny and columns for B^+ and B^- progeny (Mather, 1951). The χ^2 value was used to give a level of probability which indicates whether the markers are on the same or opposite sides of the selected marker.

Unless otherwise stated, all values deemed significant have a probability of $< 1\%$ of being due to independent segregation. There is one case in which this χ^2 value will be high for unselected markers on opposite sides of the selected marker. If the distance between

the unselected markers is large enough, progeny carrying both markers from the donor are unlikely. This situation should be obvious from cotransduction data. In most cases, the method described here gave the same result as the commonly used method of finding the rarest recombinant class and equating this class with the one requiring four crossovers, and has the advantage of providing a criterion for probability levels for the data. In addition, a few crosses did not give reasonable results based on finding the smallest class of recombinants, but the analysis by our method gave results consistent with other crosses.

RESULTS

Induction of mutants

Strains that grew on glucose but not on glucose 6-phosphate were easy to isolate after mutagenesis with ethyl methanesulphonate and enrichment with penicillin. Nitrous acid treatment gave only a small number of mutants, as might be expected from its lower efficiency. Four of these latter mutants were tested by plating them with glucose 6-phosphate as carbon source and with a crystal of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in the centre of the plate: all showed a halo of growing (and hence Uhp$^+$) colonies, and were thus not deletion mutants, even though nitrous acid is reported to lead to an increased frequency of mutants carrying deletions compared to other treatments (Schwartz & Beckwith, 1969; Alper & Ames, 1975).

Properties of strains unable to utilize glucose 6-phosphate

The strains isolated grew on solid media with glucose as carbon source, but not with glucose 6-phosphate; in liquid media, they showed normal growth rates with glucose, gluconate, fructose and glycerol. Growth on glucose 6-phosphate and fructose 6-phosphate was very slow and was probably due to hydrolysis of these hexose phosphates by phosphatases; for the same reason, the mutants grew well on glucose 1-phosphate (Dietz & Heppel, 1971*c*). Washed suspensions of these mutants took up [^{14}C]glucose 6-phosphate at initial rates much lower than similar suspensions of the parent organisms, thus confirming that the lesion observed was in hexose phosphate transport.

Mapping of the uhp region

In preliminary experiments some of the Uhp$^-$ strains were crossed by conjugation or phage-mediated transduction with a previously described *uhp* strain (Kornberg & Smith, 1969) and screened for growth on glucose 6-phosphate. Others were transduced to *pyrE*$^+$ using P1 propagated on a prototrophic strain and were again screened for growth on glucose 6-phosphate to determine the cotransduction frequency. These tests failed to bring to light any strain with a lesion in a locus different from that previously described. Strains RE21 and RE37 were therefore selected as representatives of typical *uhp* strains for determining the location of *uhp* with respect to the various markers in the vicinity of minute 81 on the *E. coli* genetic map.

These two strains were crossed with the *mtlA* strain 236 and the *mtlD* strain 239 (Table 2). Cotransduction between *mtl* and *uhp* appears to be very rare; thus, only the cross with *pyrE*$^+$ as the selected marker and strain 236 as donor could be analysed by the normal methods used for three-factor crosses. In both cases, the χ^2 value between *mtl* and *uhp* showed significant deviation at the 0·05 level. However, the rarest class of recombinants in both cases was that having the donor markers *uhp*$^+$ and *mtl*, so this value must result from the low cotransduction of the two markers rather than their location on the same side of *pyrE*. Thus

Table 2. *Crosses between pyrE uhp strains and mtl strains*

Crosses were performed as described. Unselected markers were scored for the ability to grow on appropriately supplemented plates, except that some determinations for mannitol used eosin-methylene blue plates with 50 mM-mannitol. Chi-squared values indicate the significance of the effect on inheritance of one unselected marker by inheritance of a second, as described in Methods. These values have one degree of freedom. The number in parentheses is the confidence level for acceptance of the null hypothesis that the ratios are equal.

			Recombinants which are					Cotransduction (%)		
Selection	Donor	Recipient	*uhp*$^+$*mtl*$^+$	*uhp*$^+$*mtl*	*uhp mtl*$^+$	*uhp mtl*	Total	*uhp*$^+$	*mtl*	χ^2
pyrE$^+$	236	RE21	253	4	121	25	403	64	7	15·21 (< 0·1 %)
	236	RE37	135	4	63	20	222	63	11	24·27 (< 0·1 %)
	239	RE21	142	0	146	6	294	48	2	—
	239	RE37	48	0	24	10	82	59	12	—
			pyrE$^+$*mtl*$^+$	*pyrE*$^+$*mtl*	*pyrE mtl*$^+$	*pyrE mtl*		*pyrE*$^+$	*mtl*	
uhp$^+$	236	RE21	7	0	128	0	135	5	< 1	—
	236	RE37	12	0	189	0	201	6	< 1	—
			pyrE$^+$*uhp*$^+$	*pyrE*$^+$*uhp*	*pyrE uhp*$^+$	*pyrE uhp*		*pyrE*	*uhp*	
mtl$^+$	RE21	236	269	1	13	4	287	6	2	—
	RE21	239	395	2	35	1	433	8·3	0·7	—
	RE37	236	281	0	8	0	289	3	< 1	—
	RE37	239	405	0	39	2	446	9	0·4	—

Table 3. *Crosses between pyrE uhp strains and gpsA strains*

Crosses were performed as described. To ensure good inheritance of the *gpsA* marker, strains to be transduced by phage P1 propagated on strain CY115 were grown on LB containing 10 mM-*sn*-glycerol 3-phosphate to induce the glycerol phosphate uptake system. For selection of *pyrE*$^+$ strains, bacteria were grown with glycerol as carbon source supplemented with *sn*-glycerol 3-phosphate, while inheritance of *gpsA* was tested using glucose as carbon source and no *sn*-glycerol 3-phosphate supplementation.

			Recombinants which are					Cotransduction (%)		
Selection	Donor	Recipient	*gpsA*$^+$*uhp*$^+$	*gpsA*$^+$*uhp*	*gpsA uhp*$^+$	*gpsA uhp*	Total	*gpsA*	*uhp*$^+$	χ^2
pyrE$^+$	CY115	RE21	340	280	34	64	718	14	52	13·76 (< 0·1 %)
	CY115	RE37	89	82	7	25	203	16	47	9·84 (< 1 %)
			gpsA$^+$*pyrE*$^+$	*gpsA*$^+$*pyrE*	*gpsA pyrE*$^+$	*gpsA pyrE*		*gpsA*	*pyrE*$^+$	
uhp$^+$	CY115	RE21	11	231	0	0	242	< 1	5	—
	CY115	RE37	12	233	0	0	245	< 1	5	—
			pyrE$^+$*uhp*$^+$	*pyrE*$^+$*uhp*	*pyrE uhp*$^+$	*pyrE uhp*		*pyrE*	*uhp*	
gpsA$^+$	RE21	CY115	230	3	44	12	289	19	5	37·22 (< 0·1 %)
	RE37	CY115	235	2	28	27	292	19	10	116·12 (< 0·1 %)

Table 4. *Crosses between strains* RE37 *and* CS7

Crosses were performed as described, using phage P1 grown on strain CS7 to transduce RE37. Strains able to grow on glutamate as sole carbon source were scored as gltC^c.

Selected marker and total no. of recombinants	*pyrE*$^+$		577	*uhp*$^+$		342	GltC^c		850
Unselected markers	*gltC*	*uhp*		*pyrE*	*gltC*		*pyrE*	*uhp*	
No. of recombinants	c	+	415	+	c	29	+	+	90
	c	−	49	+	+	1	+	−	7
	+	+	17	−	c	172	−	+	709
	+	−	96	−	+	140	−	−	44
Cotransduction (%)	*gltC*^{c}		80	*pyrE*$^+$		9	*pyrE*$^+$		11
	uhp$^+$		75	*gltC*^{c}		59	*uhp*$^+$		94
χ^2	*gltC–uhp*		267·308 (< 0·1 %)	*pyrE–gltC*		17·812 (< 0·1 %)	*pyrE–uhp*		0·288 (< 50 %)

the order indicated in these crosses is *mtl pyrE uhp*, in agreement with the result of Kadner & Winkler (1973).

Cronan & Bell (1974) described a mutant requiring glycerol or *sn*-glycerol 3-phosphate for growth. This lesion, designated *gpsA*, was located between *mtl* and *pyrE*. To take advantage of this locus, strains RE21 and RF37 were crossed with strain CY115, which carried the *gpsA* marker (Table 3). In the crosses with *pyrE*$^+$ as the selected marker χ^2 was significant, but the rarest class was the donor phenotype GpsA$^-$Uhp$^+$, so this value must again be due to the low cotransduction between *gpsA* and *uhp*. The order is therefore *gpsA pyrE uhp*. The crosses selected for *uhp*$^+$ showed no inheritance of *gpsA*, confirming the low cotransduction. However, the crosses selected for *gpsA*$^+$ showed measurable cotransduction of *gpsA* and *uhp*. Again, χ^2 values indicated high significance, but in this case inheritance of *uhp* enhanced that of *pyrE* which implies that *pyrE* and *uhp* are on the same side of *gpsA*. This confirms the order *gpsA pyrE uhp* because *pyrE*$^+$ *uhp* recombinants would require four crossovers. The cotransduction frequencies differed depending on which marker was selected. For example, the *pyrE–uhp* frequency was about 50 % if *pyrE*$^+$ was selected, which confirms the results of Kornberg & Smith (1969), but was only 5 % if *uhp*$^+$ was selected. This difference was also observed for *gpsA–uhp* crosses, but it was not apparent for *gpsA–pyrE*.

The *gltC*^{c} mutation described by Marcus & Halpern (1969) permits *E. coli* K12 strains to grow at 37 °C on glutamate as sole carbon source: wild-type K12 strains do not. This marker was located between *pyrE* and *tna*. Strain RE37 was therefore crossed with *gltC* ^{c}strain CS7 (Table 4). With *pyrE*$^+$ as the selected marker, highly significant χ^2 values were observed for the effect of *uhp* inheritance on *gltC* inheritance. Inheritance of *uhp*$^+$ enhanced inheritance of *gltC*^{c}, which implies that the order is *pyrE gltC uhp*. When *uhp*$^+$ was selected, similarly significant effects were seen, with inheritance of *pyrE*$^+$ enhancing that of *gltC*^{c}. This result also implies the order *pyrE gltC uhp*. Finally, when *gltC*^{c} was the marker selected, there was no significant effect of *pyrE* inheritance on *uhp* inheritance, which implies that the unselected markers are on opposite sides of *gltC*, in agreement with the other results. It is noteworthy that *gltC*^{c} and *uhp* are 94 % cotransducible, if *gltC*^{c} is selected. This cross shows unequal cotransduction frequencies in reciprocal crosses as do those involving *gpsA*.

The gene governing the activity of the enzyme tryptophanase, *tna*, is located on the *E. coli* linkage map between *gltC* and *phoS* (Bachmann *et al.*, 1976). Strain AB2147, carrying the *tna* lesion, was crossed with strain RE37 (Table 5). As expected, *tna* appears to be closer to

Table 5. *Crosses between strains* RE37 *and* AB2147

Crosses were performed as described, using phage P1 grown on AB2147 to transduce RE37. The method of Pittard & Walker (1967) was used to score Tna.

Selected marker and total no. of recombinants	*pyrE*$^+$		712	*uhp*$^+$		555
Unselected markers	*uhp*	*tna*		*pyrE*	*tna*	
No. of recombinants	+	+	442	+	+	38
	+	−	15	+	−	3
	−	+	251	−	+	388
	−	−	4	−	−	126
Cotransduction (%)	*uhp*$^+$ *tna*		64 3	*pyrE*$^+$ *tna*		7 23
χ^2	*uhp–tna*		1·250 (< 30 %)	*pyrE–tna*		5·367 (< 5 %)

Table 6. *Crosses between strains* RE37 *and* EI77

Crosses were performed as described, using phage P1 grown on the strain indicated as donor. In the crosses with EI77 as donor, selective plates were incubated at 30 °C so as not to select against inheritance of *dnaA*. Recombinants that did not grow at 42 °C were scored as DnaA$^-$.

Donor	EI77			EI77			RE37		
Recipient	RE37			RE37			EI77		
Selected marker and total no. of recombinants	*pyrE*$^+$		209	*uhp*$^+$		431	*dnaA*$^+$		431
Unselected markers	*uhp*	*dnaA*		*pyrE*	*dnaA*		*pyrE*	*uhp*	
No. of recombinants	+	+	126	+	+	51	+	+	329
	+	−	0	+	−	15	+	−	100
	−	+	77	−	+	333	−	+	2
	−	−	6	−	−	32	−	−	0
Cotransduction (%)	*uhp*$^+$ *dnaA*		60 3	*pyrE*$^+$ *dnaA*		15 11	*pyrE* *uhp*		0·5 23
χ^2	*uhp–dnaA*		6·96 (< 1 %)	*pyrE–dnaA*		11·21 (< 0·1 %)	*pyrE–uhp*		—

uhp than to *pyrE*, the cotransduction frequencies being 23 and 3 %, respectively. The χ^2 value for the effect of *uhp* inheritance on *tna* inheritance in the cross selected for *pyrE*$^+$ was not highly significant, probably due to the smaller cotransduction between *pyrE* and *tna*. The order can be deduced from cotransduction frequencies as *pyrE uhp tna*. In the cross where *uhp*$^+$ was selected, the χ^2 value showed a significant effect because of the improbability of inheritance of both *pyrE*$^+$ and *tna* from the donor (3/555 colonies tested). Again, cotransduction frequencies were not equal for the two possible selections.

A gene governing initiation of DNA synthesis, *dnaA*, has been provisionally located between *tna* and *phoS* (Wechsler & Gross, 1971). Strain EI77, carrying a temperature-sensitive lesion in *dnaA*, was crossed with strain RE37 (Table 6). There was very little cotransduction between *pyrE* and *dnaA* in either direction. As expected, cotransduction frequencies between *dnaA* and *uhp* indicate that *dnaA* is farther from *uhp* than is *tna*. Because of the low cotransduction frequencies observed, χ^2 values would not be meaningful, and were not calculated.

Strain RE21 was unexpectedly found to be *tna*. Crosses with AB2147 indicated that this *tna* allele was at the same locus as that in AB2147, so RE21 was used in crosses with CS7 to con-

Table 7. *Crosses between strains* RE21 *and* CS7

Crosses were performed as described, using P1 grown on strain CS7 to transduce RE21. Recombinants able to utilize glutamate as sole carbon source were scored as GltC^c.

Selected marker and total no. of recombinants	*pyrE*$^+$			315	*gltC*^{c}			759	*uhp*$^+$			867
Unselected markers	*gltC*	*uhp*	*tna*		*pyrE*	*uhp*	*tna*		*pyrE*	*gltC*	*tna*	
No. of recombinants	c	+	+	14	+	+	+	28	+	c	+	20
	c	+	−	105	+	+	−	31	+	c	−	14
	c	−	+	7	+	−	+	0	+	+	+	2
	c	−	−	70	+	−	−	5	+	+	−	1
	+	+	+	4	−	+	+	487	−	c	+	309
	+	+	−	9	−	+	−	147	−	c	−	75
	+	−	+	2	−	−	+	24	−	+	+	374
	+	−	−	104	−	−	−	37	−	+	−	72
Cotransduction (%)	*gltC*^{c}			62	*pyrE*$^+$			8·4	*pyrE*$^+$			4·3
	uhp$^+$			42	*uhp*$^+$			91·3	*gltC*^{c}			48·3
	tna$^+$			8·6	*tna*$^+$			71·0	*tna*$^+$			81·4
	gltC^c *uhp*$^+$			38	*pyrE*$^+$ *uhp*$^+$			7·8	*pyrE*$^+$ *gltC*^{c}			3·9
	gltC^c *tna*$^+$			6·7	*pyrE*$^+$ *tna*$^+$			3·7	*pyrE*$^+$ *tna*$^+$			2·5
	uhp$^+$ *tna*$^+$			5·7	*uhp*$^+$ *tna*$^+$			67·8	*gltC*^{c} *tna*$^+$			38·0
χ^2	*gltC–uhp* 75·400 (< 0·1 %)				*pyrE–uhp* 0·069 (< 70 %)				*pyrE–gltC* 27·734 (< 0·1 %)			
	gltC–tna 3·040 (< 10 %)				*pyrE–tna* 42·165 (< 0·1 %)				*pyrE–tna* 12·151 (< 0·1 %)			
	uhp–tna 7·438 (< 1 %)				*uhp–tna* 25·241 (< 0·1 %)				*gltC–tna* 3·610 (< 5 %)			

firm the order between *gltC*, *uhp* and *tna*, since all three would be present in the same cross. The results are shown in Table 7. In the selection for *pyrE*$^+$, the cotransduction frequencies decreased in the order *gltC* > *uhp* > *tna*. Further, *gltC* and *uhp* had a significant effect on each other, as did *uhp* and *tna*. The order indicated is *pyrE gltC uhp tna*. In the selection for *gltC*^{c}, the order of cotransduction frequencies of unselected markers subsequently scored is *uhp* > *tna* > *pyrE*. Double cotransductions indicated that, of these three markers, *uhp* and *tna* are closest and *pyrE* and *tna* are farthest apart, which again supports the order *pyrE uhp tna*. The lack of effect of *pyrE* on *uhp* indicates that *gltC* falls between these markers, so the overall order measured in this manner is also *pyrE gltC uhp tna*. In the selection for *uhp*$^+$, double cotransduction frequencies give the order *pyrE gltC tna*, and *uhp* must be between *gltC* and *tna*, because of cotransduction frequencies and the lack of significant effects between these markers.

Fine structure mapping

To estimate the extent of the *uhp* region and to get some idea of the relative location of mutations giving rise to constitutive and negative mutants, a series of crosses between negative strains or between negative and constitutive strains was done. The order of the lesions can sometimes be deduced in such a series of crosses, using results from reciprocal crosses. The strains used for this series were all *pyrE*, so the most convenient selection was for *pyrE*$^+$. Accordingly each strain was transduced to *pyrE*$^+$ using phage grown on wild-type K10 cells. Strains were tested for retention of the original *uhp* mutations. These *pyrE*$^+$ strains were then used as donors in crosses in various combinations with the *pyrE* strains (Table 8). In all cases, recombinants of the phenotype expected from crossovers between the lesions were observed, indicating that the lesions were at different sites.

Table 8. *Fine structure mapping of uhp and uhp^c strains*

Transductions were performed as described, selecting for growth in the absence of uracil in all cases. For crosses between negative strains, Pyr$^+$ selective plates were replicated on to plates containing glucose 6-phosphate as sole carbon source and the total number of Pyr$^+$ and Pyr$^+$Uhp$^+$ recombinants was counted. For crosses between AT2243-11^c or AT2243-11^{c}U and the negative strains, Pyr$^+$ transductants were transferred using sterile toothpicks on to plates containing either glucose 6-phosphate or fructose 1-phosphate as sole carbon source. By comparison of these plates, Uhp$^+$ (not Uhp^c) strains were scored. The value of χ^2 is calculated from the 2×2 contingency table whose rows are the two reciprocal crosses, and whose columns are recombinants (Uhp$^+$) versus non-recombinants (Uhp$^-$ or Uhp^c) for the region between the two mutations. This value has one degree of freedom.

		Recombinants			
		Number		Percentage	
Donor	Recipient	Pyr$^+$	Uhp$^+$	Uhp$^+$/Pyr$^+$	χ^2
RE21U	RE30	562	22	3·9	4·26 (< 5 %)
RE30U	RE21	379	6	1·6	
RE21U	RE37	884	18	2·0	7·04 (< 1 %)
RE37U	RE21	759	4	0·5	
RE30U	RE37	1427	14	1·0	0·00013 (< 99 %)
RE37U	RE30	1740	17	1·0	
AT2243-11^{c}U	RE21	941	5	0·5	0·98 (< 50 %)
RE21U	AT2243-11^c	984	9	0·9	
AT2243-11^{c}U	RE37	854	9	1·1	3·94 (< 5 %)
RE37U	AT2243-11^c	830	19	2·3	

DISCUSSION

From the results presented here, it is possible to construct a detailed map of the *E. coli* genome in the vicinity of minute 81 (Fig. 1). The *uhp* gene lies between *gltC* and *tna* and, from the cotransduction frequencies, appears to be quite close to *gltC*, possibly adjacent. These results are in substantial agreement with those of Kadner & Winkler (1973), who placed *uhp* between *pyrE* and *bgl*. There are some small discrepancies: Kadner & Winkler (1973) reported the cotransduction frequency of *pyrE* and *uhp* to be between 29 and 46 %, whereas we find a range of 42 to 75 %. This may simply be a strain difference, or could result from slightly different procedures for the transduction experiments. Also, the striking asymmetry in cotransduction frequencies, which depends on the end of the interval at which the selected marker lies, does not appear to have been observed previously.

This lack of reciprocity in the cotransduction frequencies appears to be most pronounced in the region of *uhp*, and was manifested by all cotransductions with *uhp*. However, it was also obtained in several cotransductions which do not involve *uhp* (as, for example, the cotransductions *pyrE–gltC* and *gltC–dnaA*) and so is not associated uniquely with *uhp*. In most cases, the cross with the selected marker counterclockwise from the unselected one gave the higher frequency, but in the case of *uhp–dnaA*, the reverse was observed. In all cases involving *uhp*, the frequency observed in the cross where it was the selected marker was lower. This was not due to reversion of *uhp*, since no revertants grew on the control plates; moreover, the same result was obtained with two independent *uhp* mutants. The origin of replication of the chromosome is in the region between *bgl* and *mtl* (Masters, 1975) and thus very close to *uhp*: this location may lead to favoured sites of chromosome breakage, which could explain the results.

Construction of a fine structure map on the basis of the results presented in Table 8 poses some difficulties. The placement of the negative lesions is relatively easy since the orders

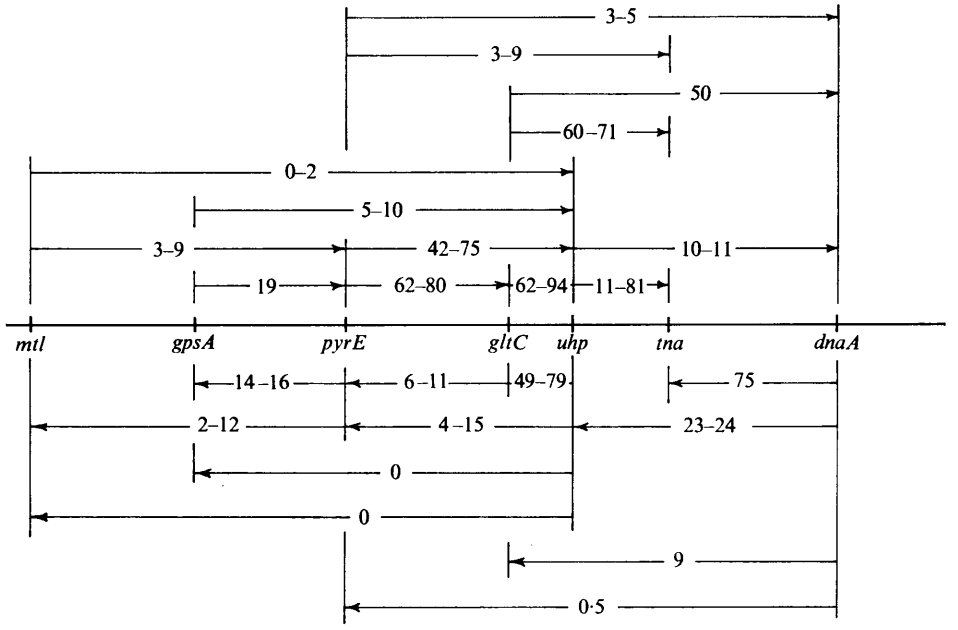

Fig. 1. Map of the *E. coli* chromosome in the region of minute 81. The order indicated is that deduced from the crosses presented although, for the sake of brevity, no data are given in this paper for the transductions *dnaA–tna*, *dnaA–gltC* and *gltC–dnaA*. The figures are the contransduction frequencies observed. Where a range is indicated, the numbers are the maximum and minimum for all crosses. Frequencies above the line had the marker on the left as the selected marker, those below had the marker on the right.

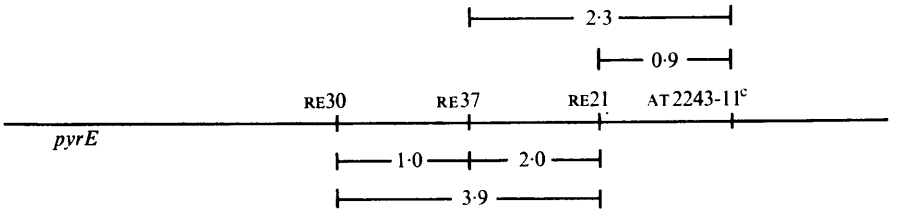

Fig. 2. Fine structure map of the *uhp* region. Negative lesions are ordered according to the data from the reciprocal crosses where consistent with recombination frequencies. The constitutive allele is located using the observed recombination frequencies. Distances indicated are the highest observed percentage of *pyrE*$^+$ recombinants which were recombinant in the region between the lesions.

deduced from the differences between results from reciprocal crosses are consistent with the distance based on recombination frequencies. However, the *uhp*^{c} lesion cannot be placed without disregarding either the distance data or the order data. It seems more likely that the distance data are correct, especially since the significance of the differences between the reciprocal crosses is low or non-existent. Using the distance data, one obtains the map shown in Fig. 2. The distances are nearly additive, and *uhp*^{c} is on the distal side of the *uhp* region from *pyrE*, as proposed by Kadner (1973). Taking the minimum distance, or the average, rather than the maximum, the additivity is even better. Negative interference could lead to equal frequencies in the reciprocal crosses, and would be more pronounced with a relatively distant marker, such a *pyrE*. Use of a closer marker such as *gltC* could resolve this problem.

The capable technical assistance of Kathleen Brown, Kathal Bales and Theresa Mason is gratefully acknowledged. We also thank Drs B. Bachmann, J. E. Cronan, Jr, Y. S. Halpern, E. C. C. Lin, E. J. Murgola, and J. L. Rosner for generous gifts of bacteria and bacteriophage. Dr M. C. Jones-Mortimer suggested the method of analysis of the crosses, and aided us with much helpful discussion.

This work was supported in part by grant number B/SR/72462 from the Science Research Council. Part was done while R.C.E. was supported by National Institutes of Health postdoctoral fellowship 5FO2 AM47162-02.

This is journal article J-3503 of the Oklahoma Agricultural Experiment Station.

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