Journal of General Microbiology (1983), **129**, 75–84. *Printed in Great Britain*

Polymorphism in *Escherichia coli*: *rtl atl* and *gat* Regions Behave as Chromosomal Alternatives

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(*Received 13 April 1982*)

Amongst forty wild strains of *Escherichia coli*, sixteen utilized galactitol (as did K12) and seven utilized ribitol (as did C) of which six utilized D-arabitol; none utilized all three polyols.

Transduction of genes for ribitol utilization (*rtl*$^+$) to strains able to utilize galactitol (*gat*$^+$), whether K12 or wild strains, using wild strains and *E. coli* C as donors always resulted in loss of the galactitol phenotype. The genes for D-arabitol use (*atl*$^+$) were always cotransduced with *rtl*$^+$ in interstrain crosses. We confirm and extend the mapping of *gat*$^+$ (Lengeler, 1977) and *rtl*$^+$ *atl*$^+$ (Reiner, 1975) in their respective hosts, K12 and C, by showing both regions to be 50% cotransducible with *metG* and 3% cotransducible with *fpk*. In reciprocal transductions, *gat*$^+$ replaced *rtl*$^+$ *atl*$^+$. In partial diploids, *rtl*$^+$ *atl*$^+$ and *gat*$^+$ regions did not interfere with each other's expression.

Transfer of *rtl*$^+$ from an Rtl$^+$ Atl$^-$ donor by R plasmid (pE10)-mediated conjugation, gave Gat$^-$ transconjugants of K12 in which *rtl*$^+$ and a kanamycin resistance gene were 100% cotransducible in the *metG* region of the chromosome.

It is suggested that the *rtl*$^+$ *atl*$^+$ and *gat*$^+$ genes (or parts of them) act as alternative, or mutually exclusive, regions in the chromosome. Possible reasons for the existence of alternative characters are discussed.

INTRODUCTION

An enormous amount of natural variation occurs in *Escherichia coli* and other members of the *Enterobacteriaceae*. As the first stage in studying the genetic basis of this variation, characters were chosen for which *E. coli* K12 is negative and for which some wild strains are positive. Tests were then made to determine whether the characters were transferrable to K12, and to determine whether their genes took constant chromosomal locations in K12. For ease of selection in genetic crosses, the characters of first choice were those concerned with utilization of carbohydrates. So far, sucrose utilization (Alaeddinoglu & Charles, 1979; Hill, 1980; Hill & Charles, 1980) and L-sorbose utilization (Woodward, 1980; Woodward & Charles, 1980, 1982) have been studied. The genes for each character took a characteristic locus in K12. A study of ribitol, D-arabitol and galactitol utilization is now reported. These characters proved to be of special interest in that the genes for ribitol and D-arabitol utilization, on the one hand, and those for galactitol utilization on the other, behaved as mutually exclusive alternatives in the chromosome.

Ribitol and D-arabitol are used by 85% of *Klebsiella* strains (Cowan & Steel, 1974) and 10% of *E. coli* strains (Edwards & Ewing, 1972). Amongst laboratory strains, *E. coli* C utilizes ribitol and D-arabitol but K12 and B do not (Reiner, 1975). Strains able to use ribitol and D-arabitol mutate to use xylitol (Mortlock *et al.*, 1965; Wu *et al.*, 1968) and this has been of interest in the study of enzyme evolution (Inderlied & Mortlock, 1977) and gene duplication (Rigby *et al.*, 1974). The *rtl*$^+$ *atl*$^+$ genes were transduced from *E. coli* C into K12 (Reiner, 1975) as were the *rbt*$^+$ *dal*$^+$ genes

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0022-1287/83/0001-0519 $02.00

from *K. aerogenes* (Rigby *et al.*, 1976). In both cases the genes, although distinct, showed 100% cotransduction and took a position near *metG*, at 46 min on the current linkage map (Bachmann & Low, 1980).

Catabolism of ribitol and D-arabitol in *K. aerogenes* and *E. coli* depends upon different inducible operons, each coding for a dehydrogenase (*rbtD, dalD* and *rtlA, atlA*) and a kinase (*rbtK, dalK* and *rtlA, atlB*). The genes of the two operons are closely linked and lie in mirror image arrangements: *dalK dalD dalC rbtB dalB rbtC rbtD rbtK* in *K. aerogenes* (Charnetzky & Mortlock, 1974*a, b, c*) and *rtlB rtlA rtlC atlC atlA atlB* in *E. coli* (Scangos & Reiner, 1978). The genetic and biochemical similarities of ribitol and D-arabitol utilization in *E. coli* and *K. aerogenes* led to the suggestion that *Klebsiella* was a likely source of the ribitol and D-arabitol genes in *E. coli*, if the genes originated extragenerically (Reiner, 1975). The fact that stable integration of *Klebsiella* genes into the *E. coli* chromosome occurs after R-plasmid-mediated conjugation (Cannon *et al.*, 1974) and after P1 transduction (Rigby *et al.*, 1976) may support this speculation.

Catabolism of galactitol in *E. coli* depends upon an inducible operon coding for a phosphoenolpyruvate-dependent phosphotransferase enzyme II (*gatA*) and a dehydrogenase (*gatD*); the genes are closely linked in the order *gatC gatA gatD* and map near *metG* at 46 min on the linkage map (Lengeler, 1975*a*, 1977; Lengeler & Steinberger, 1978).

METHODS

The methods used followed Alaeddinoglu & Charles (1979), except when stated otherwise.

Lysogenization and transduction by P1clr100KM. These were as described by Goldberg *et al.* (1974) and as modified by Woodward & Charles (1982). When appropriate, P1*clr100*KM lysogens of wild strains were used as recipients in transduction. Recipients were grown at 30 °C with vigorous agitation in T2 broth supplemented with kanamycin (12·5 mg l^{-1}). For transduction, 10^9 bacteria, 10^9 phage and 0·1 ml $CaCl_2$ (0·1 M) were mixed and adsorption was at 30 °C for 25 min.

Gene symbols and map distances. These follow Bachmann & Low (1980), but the positive, rather than the wild-type alleles are indicated by a plus sign. Except in Table 1, genes are shown in their order on the linkage map starting at 0 min. Following Reiner (1975), the symbols rtl^+ and atl^+ represent genes, occurring in some wild strains, which confer the abilities to utilize ribitol and D-arabitol, respectively. The degree of homology between rtl^+ atl^+ genes from *E. coli* C and from other wild strains is not known.

Strain designations and genotypes. These are shown in Table 1. Strain MW23 was obtained by mutagenesis of strain AB1621; the new allele *ato-23* was similar to the *atoA* and *atoB* alleles (Pauli & Overath, 1972) in blocking butyrate utilization and in being 50% cotransducible with *nalA*. Strain MW39 was made by mutagenesis of strain AB1621; the new allele *gat-39* was similar to *gatC, gatA* and *gatD* alleles (Lengeler, 1977) in blocking galactitol utilization and in mapping at 46 min in time-of-entry experiments.

RESULTS

Acquisition of rtl^+ atl^+ genes caused K12 to become Gat^-

Of forty strains tested, seven utilized ribitol and D-arabitol, one utilized ribitol only, and none used D-arabitol only. Treatment with sodium lauryl sulphate (Tomoeda *et al.*, 1968) or acridine orange (Salisbury *et al.*, 1972) did not cause loss of the ribitol and D-arabitol characters, suggesting that the characters were not due to plasmids. P1kc did not give plaques on the wild strains. The method of Goldberg *et al.* (1974), modified by Woodward & Charles (1982), gave P1*clr100*KM lysogens of each ribitol-positive strain. The lysogens were induced at 42 °C. Lysates of RK11, RK52, RK95 and RK120 gave about 10^9 plaques ml^{-1} when assayed on restrictionless mutant 5K, and were used to transduce K12 recipients (Table 2). Control selection for $ArgA^+$ and $MetB^+$ gave frequencies of transduction and cotransduction comparable with those for K12 to K12 crosses (McConville, 1977). Rtl^+ transductants occurred with frequencies similar to Xyl^+ transductants. Lysates of RK1, RK6, RK65 and RK79 contained about 10^4 phage ml^{-1}, insufficient for transduction.

Reiner (1975) showed that genes rtl^+ and atl^+, conferring ability to utilize ribitol and D-arabitol, were 95% cotransducible within *E. coli* C, and 100% cotransducible when transduced

Table 1. *Escherichia coli strains*

Strain	Genotype	Source or reference
AB1621	F⁻ *ara lac tsx gal xyl mtl glpD thiA*	Adelberg *et al.* (1965)
AT2243-11^{c}-25	F⁻ *tonA22 relA1 fpk-1 uhp-1 pyrE41 metB1*	Ferenci & Kornberg (1973)
JC1553	F⁻ *leu his recA rpsL malA argG metB*	Clark & Marguiles (1965)
JC5466	F⁻ *trp his recA rpsL*	Clark & Marguiles (1965)
KL144	F⁻ *tonA2 tsx relA aroC purF recA rpsL*	Low (1973)
MLM161	F⁻ *his rpsL mal mtl ilv metB glpK argH*	McConville & Charles (1979)
N1332	F⁻ *proB lacZ118 trpA605 nalA rpsL recA200ts metE*	G. I. Lloyd, Nottingham
SB1803	F⁻ *thr-1 leu-6 proA2 ara lacY supE44 galK2 hisC3 metG83 rpsL mtl xyl*	Blumenthal (1972)
5K	F⁻ *thr-1 leu-6 lacY tonA21 hsdR514 rpsL*	Colson *et al.* (1965)
AB2575	Hfr *tsx ilv thiA*	Pittard *et al.* (1963)
GA312	VHfr *sac*⁺ *thr-1 leu-6 rpsL*	Alaeddinoglu & Charles (1979)
GA997	Hfr *sac*⁺ *dapC relA thi-1*	Alaeddinoglu & Charles (1979)
KL983	Hfr *lacY mglP1 xyl-7*	Low (1973)
R4	Hfr *relA1 metB1*	Reeves (1959)
44	Hfr *galT23 argS41 argA42 argR40 ilv-282*	Cooper *et al.* (1969)
MW16	*rtl*⁺ *atl*⁺ transductant of strain Hfr44, donor MW111	This work
MW23	*ato-23* mutant of strain AB1621 made by MNNG mutagenesis	This work
MW39	*gat-39* mutant of strain AB1621 made by MNNG mutagenesis	This work
MW81	*rtl*⁺ *atl*⁺ *recA purF aroC argH thi-1*	This work
MW111–3	*rtl*⁺ *atl*⁺ transductants of strain AB1621, donor RK11	This work
MW521–3	*rtl*⁺ *atl*⁺ transductants of strain AB1621, donor RK52	This work
MW591–3	*rtl*⁺ *atl*⁺ transductants of strain AB1621, donor RK95	This work
MW1201–3	*rtl*⁺ *atl*⁺ transductants of strain AB1621, donor RK120	This work
MW5651–4	*rtl*⁺ transconjugants of strain 5K, donor RK65pE10	This work
RK1–120	Wild strains isolated from the River Kennet	This work

Table 2. *Transduction of genes from E. coli wild strains into K12*

Donor	Phage titre*	Recipient	Selected allele	Transductants per 10^7 phage	Number scored	Unselected allele	Cotransduction frequency (%)
RK11	$6{\cdot}3 \times 10^8$	MLM161	*argH*⁺	3·7	200	*metB*⁺	37
			metB⁺	4	200	*argH*⁺	44
		AB1621	*rtl*⁺	1	107	*atl*⁺	100
			xyl⁺	3·7	—	—	—
RK52	$8{\cdot}7 \times 10^8$	MLM161	*argH*⁺	7·5	244	*metB*⁺	21
			metB⁺	6	326	*argH*⁺	16
		AB1621	*rtl*⁺	4	407	*atl*⁺	100
			xyl⁺	9	—	—	—
RK95	$1{\cdot}4 \times 10^9$	MLM161	*argH*⁺	15	500	*metB*⁺	23
			metB⁺	22	500	*argH*⁺	26
		AB1621	*rtl*⁺	1·2	117	*atl*⁺	100
			xyl⁺	8	—	—	—
RK120	$1{\cdot}2 \times 10^9$	MLM161	*argH*⁺	8	500	*metB*⁺	50
			metB⁺	8	500	*argH*⁺	32
		AB1621	*rtl*⁺	0·8	81	*atl*⁺	100
			xyl⁺	6	—	—	—

* phage P1*clr100*KM

from *E. coli* C to K12. All the 712 *rtl*⁺ transductants in Table 2 proved to be *atl*⁺, contrasting with their recipient parent AB1621 which, like other K12 derivatives, did not use D-arabitol.

The possibility was examined that when bacteria acquired the *rtl*⁺ region of the chromosome they might lose a corresponding region which coded for other characters. A K12 *rtl*⁺ transductant from each interstrain cross was tested for gain or loss of ability to use any of 212

substances as carbon and energy sources. One difference was observed: all hybrids had lost the ability to use galactitol. About 4000 *rtl*$^+$ transductants have now been tested: all had gained the ability to use D-arabitol and lost the ability to use galactitol, regardless of whether the donor was RK11, RK52, RK95 or RK120.

Genes rtl$^+$*, atl*$^+$ *and gat*$^+$ *mapped near metG*

The *rtl*$^+$ genes were mapped by interrupted conjugation. Three *rtl*$^+$ transductants from each of the original crosses, designated MW111 to MW113, MW521 to MW523, MW591 to MW593 and MW1201 to MW1203 (from donors RK11, RK52, RK95 and RK120, respectively) were used as donors to transduce HfrGA997 and HfrGA312 to *rtl*$^+$. The *rtl*$^+$ derivatives of these Hfr strains transferred the *rtl*$^+$ genes after 30 min and 75 min, respectively, indicating a locus between *his* (44 min) and *sac* (50·5 min). Using a *rtl*$^+$ derivative of GA997 as donor and SB1803 (*his metG rpsL*) as recipient, selection was made separately for *his*$^+$, *rtl*$^+$, *metG*$^+$ (47 min) and *sac*$^+$ transconjugants. The genes were transferred at intervals of 4, 1 and 2 min, respectively. The results placed *rtl*$^+$ in the 46 min region, which contains three known markers (Bachmann & Low, 1980). Mapping of markers in the 44–48 min region has been mainly by conjugation (B. J. Bachmann, personal communication). Tests were made for cotransduction of *rtl*$^+$ with *his, mglP1, fpk, metG, ato* and *nalA* (Table 3), using phage P1kc and donor MW111. Selection was made for Rtl$^+$ and for the other wild-type alleles when possible, but MglP1$^+$ and NalA$^+$ were not selectable. The allele *mglP1* reverted with a frequency of about 10^{-5} and distinction between transductants and revertants was not possible; *nalA*$^+$ conferred sensitivity to nalidixic acid. The *rtl*$^+$ region was cotransduced with *metG* (53%) and with *fpk*$^+$ (3%), but not with *his, mglP1, ato* or *nalA*. Further mapping was not attempted. About 2000 *rtl*$^+$ transductants from the mapping experiments were streaked on D-arabitol and galactitol; all used D-arabitol and none used galactitol.

Lengeler (1975*a, b*) mapped the galactitol utilization genes (*gat*$^+$) by time-of-entry at about 46 min, but did not report their order with respect to nearby markers. According to Lengeler (1977), the original K12 from Lederberg's collection, most F$^-$ and a few Hfr strains use galactitol at 30 °C and below, but some do not. Tests showed that some of our marker strains used galactitol at 30 °C (*gat*$^+$) and others did not (*gat*), and this variation was used to map the *gat*$^+$ region. In time-of-transfer experiments GA997 *gat*$^+$ was mated with MLM161 (*his gat*) and selection was made separately for His$^+$, Gat$^+$ and Sac$^+$. The genes were transferred after 27, 25 and 19 min, respectively, confirming Lengeler's report. Amongst the strains available with nearby markers, SB1803 (*metG*) and MW23 (*ato*) were *gat*$^+$, and MLM161 (*his*) and AT2243-11^c-25 (*fpk*) were *gat*; Table 4 shows that *fpk*$^+$ was 3% cotransducible with *gat*$^+$, and a *gat* allele was 49% cotransducible with *metG*$^+$.

Partial diploids expressed gat$^+$ *and rtl*$^+$ *atl*$^+$

Introduction of the *rtl*$^+$ *atl*$^+$ region caused Gat$^+$ bacteria to become Gat$^-$ either because their *gat*$^+$ genes were replaced, or disrupted, or blocked in expression. Partial diploids were prepared,

Table 3. *Cotransduction frequencies in the rtl region (donor strain MW111)*

Recipient	Selected allele	Transductants per 10^7 phage	Number scored	Unselected allele	Cotransduction frequency (%)
MLM161	*his*$^+$	22	500	*rtl*$^+$	0
	rtl$^+$	9·5	500	*his*$^+$	0
KL983	*rtl*$^+$	6	400	*mglP1*$^+$	0
AT2243-11^c-25	*fpk*$^+$	28·6	540	*rtl*$^+$	0
	rtl$^+$	7	250	*fpk*$^+$	3
SB1803	*metG*$^+$	4	200	*rtl*$^+$	54
	rtl$^+$	4	200	*metG*$^+$	81
MW23	*ato-23*$^+$	7	450	*rtl*$^+$	0
	rtl$^+$	6	320	*ato-23*$^+$	0
N1332 *nalA*$^+$	*rtl*$^+$	7	400	*nalA*	0

Table 4. *Cotransduction frequencies in the gat region*

Donor	Recipient	Selected allele	Transductants per 10^7 phage	Number scored	Unselected allele	Cotransduction frequency (%)
AB1621	MLM161	*his*$^+$	21	500	*gat*$^+$	0
		gat$^+$	9	500	*his*$^+$	0
AB1621	AT2243-11^c-25	*fpk*$^+$	24	500	*gat*$^+$	0
		gat$^+$	5	230	*fpk*$^+$	3
MW39	SB1803	*metG*$^+$	4	200	*gat*	49
MW39	MW23	*ato-23*$^+$	7	500	*gat*	0

to show whether *gat*$^+$ genes were expressed in the presence of the *rtl*$^+$ *atl*$^+$ region. Hfr44 was transduced to *rtl*$^+$ *atl*$^+$ using donor MW111. One transductant, MW16, was purified and shown to utilize ribitol and D-arabitol but not galactitol. It transferred *rtl*$^+$ *atl*$^+$ after about 100 min, a little after *his*, consistent with a locus at 46·5 min. F′ *rtl*$^+$ *atl*$^+$ plasmids were obtained from conjugation between MW16 and recipient JC1553 (*his gat*$^+$ *recA rpsL*). Selection for His$^+$ Rtl$^+$, 20 min after mixing gave about 200 transconjugants from plating 10^6 donors and 10^7 recipients; all utilized galactitol, ribitol and D-arabitol.

F′ plasmids carrying *gat*$^+$ were obtained by mating Hfr44 (*his*$^+$ *gat*$^+$) with MLM161 (*his gat rpsL*), selection being made for His$^+$ Gat$^+$ transconjugants after 20 min conjugation. About 200 His$^+$ Gat$^+$ transconjugants were obtained. One was tested in conjugation with MW81 (*rtl*$^+$ *atl*$^+$ *recA*), selection being made for Gat$^+$ recipients. Mating mixtures contained 10^6 donors and 10^7 recipients ml^{-1}; one in five donors transferred their F′ *his*$^+$ *gat*$^+$ plasmids. About 1000 Gat$^+$ transconjugants were purified by streaking three times on complete medium; all grew as well as Gat$^+$ and Rtl$^+$ Atl$^+$ haploids when streaked on galactitol, ribitol and D-arabitol media.

The experiments with partial diploids suggested that the loss of the Gat$^+$ character on introduction of the *rtl*$^+$ *atl*$^+$ region was due to replacement of the *gat*$^+$ genes, or interruption of an operon by insertion of incoming DNA. If the damage to the *gat*$^+$ genes was small, it was possible that *rtl*$^+$ *atl*$^+$ hybrids might be able to revert to Gat$^+$ by genetic rearrangement, or by loss of the *rtl*$^+$ *atl*$^+$ genes. Also, sufficient of the *gat*$^+$ DNA sequence might remain in hybrids for them to provide, in transduction, the DNA sequences required to restore some of the *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG)-induced *gat* mutants to Gat$^+$. Transduction tests, and tests with common mutagens, failed to give Gat$^+$ mutants from *rtl*$^+$ *atl*$^+$ hybrids.

Wild strains utilizing galactitol did not use ribitol or D-*arabitol*

If the *rtl*$^+$ *atl*$^+$ region and the *gat*$^+$ region were alternatives in the chromosome, then wild strains able to utilize all three substrates might not occur. Forty wild strains and strains B and C were streaked onto galactitol, ribitol and D-arabitol media. Seven strains, including *E. coli* C, utilized ribitol and D-arabitol, and one utilized ribitol alone; none of these utilized galactitol. Fourteen grew well on galactitol at 30 °C giving colonies of 2 mm diameter within 4 d; one gave translucent colonies of 0·5 mm diameter within 12 d and *E. coli* B mutated to use galactitol; none used ribitol or arabitol. The remaining strains used neither galactitol, ribitol nor D-arabitol.

Wild strains transduced to Rtl$^+$ *Atl*$^+$ *became Gat*$^-$*, and vice versa*

To test whether *rtl*$^+$ *atl*$^+$ genes were expressed in *gat*$^+$ wild strains, F′ *rtl*$^+$ *atl*$^+$ plasmids were used. JC5466 (*trp his recA*/F′ *rtl*$^+$ *atl*$^+$ *his*$^+$) was incubated with *gat*$^+$ strains and selection made for transconjugants able to utilize ribitol. They occurred with frequencies ranging from one per 50 donors to one per 10^8 donors in 12 of the 14 crosses; 10 transconjugants from each were tested by streaking and grew well on ribitol, D-arabitol and galactitol media.

In order to transduce *rtl*$^+$ *atl*$^+$ genes into Gat$^+$ wild strains, P1*clr100*KM lysogens of wild strains were first prepared to serve as recipients. Lysogens of 10 Gat$^+$ wild strains (RK strains 2, 19, 20, 42, 53, 87, 91, 99, 101 and 107) from 16 tested were obtained using the modified method of Woodward & Charles (1982). Phage P1kc grown on MW111 was used to transduce the lysogens, selection being made for Rtl$^+$ transductants. For every 10^9 infecting particles derived from

MW111, RK2 gave 3 *rtl*$^+$ transductants, RK19 gave 1, RK42 gave 42, RK91 gave 117, and the other 6 strains gave none. In these experiments 489 *rtl*$^+$ transductants were obtained; all utilized D-arabitol and none utilized galactitol.

To check that *gat*$^+$ genes were expressed in *rtl*$^+$ *atl*$^+$ bacteria, JC5466 (*trp his recA*/F′ *his*$^+$ *gat*$^+$) was incubated with wild strains, RK1, RK6, RK11, RK52, RK65, RK79, RK95 and RK120, and selection made for Gat$^+$ transconjugants. They were obtained from each mating with frequencies between one per 10^6 and one per 10^8 donors. Ten transconjugants from each were tested; they utilized galactitol, ribitol and D-arabitol.

P1kc phage from AB1621 (*gat*$^+$) was used to transduce MW111, *E. coli* C and P1*clr100*KM lysogens of Rtl$^+$ Atl$^+$ wild strains, selection being made for Gat$^+$. MW111, *E. coli* C, RK1, RK95 and RK120 gave transductants, but RK6, RK11, RK52, RK65 and RK79 did not. Of 485 Gat$^+$ transductants none utilized ribitol or D-arabitol.

Phage suspensions were also prepared by induction of P1*clr100*KM lysogens of Gat$^+$ wild strains. Those from RK4, RK19 and RK20 contained at least 10^7 phage ml^{-1}, which were sufficient for use in transduction, but those from seven other Gat$^+$ lysogens contained only 10^3–10^5 ml^{-1}. Transductions with MW111 as recipient were successful. Of almost 1000 Gat$^+$ transductants none utilized either ribitol or D-arabitol. Transductions with Rtl$^+$ Atl$^+$ P1*clr100*KM lysogenic wild strains as recipients failed.

Transfer of the Rtl$^+$ *genes from RK65 (Rtl*$^+$ *Atl*$^-$*) by conjugation*

Amongst eight wild strains which utilized ribitol, RK65 was unique in that it did not utilize D-arabitol. Although readily lysogenized by P1*clr100*KM, it gave insufficient phage for transduction. Plasmid pE10 was used for the conjugative transfer of chromosomal *sac*$^+$ genes from wild strains to K12 by Alaeddinoglu & Charles (1979). Following their methods, pE10 was transferred to RK65 and the bacteria were grown overnight with strain 5K, which lacks a restriction endonuclease. Selections were then made for Rtl$^+$, Thr$^+$ and Leu$^+$ transconjugants, with streptomycin selecting against the donor. Four Rtl$^+$ colonies were obtained from two matings (about one per 2×10^9 donors); Thr$^+$ and Leu$^+$ colonies were not obtained. Bacteria from the Rtl$^+$ colonies carried the genetic markers of 5K except that they utilized ribitol and were resistant to kanamycin, chloramphenicol, neomycin, ampicillin and tetracycline. The hybrids were given the symbols MW5651–MW5654.

The four 5K (*rtl*$^+$) hybrids and JC1553 (pE10) were treated with sodium lauryl sulphate which causes the loss of pE10 from host bacteria (Alaeddinoglu & Charles, 1979). The hybrids lost neither the ability to use ribitol, nor resistance to kanamycin, but they lost resistance to chloramphenicol, neomycin, ampicillin and tetracycline *en bloc* from 6% of treated bacteria. JC1553 (pE10) lost all five resistances, kanamycin included, *en bloc* from 5% of treated bacteria. It seemed that pE10 or a derivative was present in each hybrid. If *rtl*$^+$ had become inserted into pE10, it should enable the transfer of *rtl*$^+$ to *recA* recipients. This was tested using the four hybrids as donors and AB1621 (*recA*$^+$) and KL144 (*recA*) as recipients. Selections were made for Rtl$^+$ transconjugants, for transconjugants resistant to both kanamycin and chloramphenicol, and for Rtl$^+$ transconjugants resistant to kanamycin (Table 5); selection against the donor was achieved by omitting threonine and leucine from the medium.

Transconjugants occurred on ribitol minimal medium with and without kanamycin at a frequency of about one per 2×10^7 donors, but only when the recipient was *recA*$^+$. One hundred of each class of transconjugants, selected for *rtl*$^+$ and *rtl*$^+$ Kan^R (800 in total), were tested and all were resistant to kanamycin, chloramphenicol, neomycin, tetracycline and ampicillin.

Selection for kanamycin and chloramphenicol resistance gave about one transconjugant per 50 donors with both *recA*$^+$ and *recA* recipients; 2000 were tested and all were unable to use ribitol but were resistant to neomycin, tetracycline and ampicillin. This was evidence that the four hybrids harboured intact and autonomously-transmissible pE10, which did not carry the *rtl*$^+$ genes.

The failure to isolate Rtl$^-$ strains by treatment with sodium lauryl sulphate and the high frequency of transfer of Rtl$^+$ to *recA*$^+$ but not *recA* recipients, suggested that the *rtl*$^+$ genes were chromosomal in the four hybrids. To test this, *rtl*$^+$ was transduced from them into Hfr strains

Table 5. *Conjugation experiments with 5K (rtl⁺ Kan^R) hybrids as donors*

Donor	Recipient	Frequency of transfer per donor when selection was made for: *rtl*⁺	*rtl*⁺ Kan^R*	Kan^R, Cam^R*
MW5651	AB1621	$6{\cdot}3 \times 10^{-7}$	$6{\cdot}1 \times 10^{-7}$	$3{\cdot}4 \times 10^{-2}$
	KL144 *recA*	0	0	$2{\cdot}7 \times 10^{-2}$
MW5652	AB1621	$7{\cdot}4 \times 10^{-7}$	$7{\cdot}1 \times 10^{-7}$	$1{\cdot}7 \times 10^{-2}$
	KL144 *recA*	0	0	$1{\cdot}7 \times 10^{-2}$
MW5653	AB1621	$3{\cdot}2 \times 10^{-7}$	$3{\cdot}2 \times 10^{-7}$	$8{\cdot}2 \times 10^{-2}$
	KL144 *recA*	0	0	$4{\cdot}3 \times 10^{-2}$
MW5654	AB1621	$7{\cdot}4 \times 10^{-7}$	$6{\cdot}0 \times 10^{-7}$	$6{\cdot}0 \times 10^{-2}$
	KL144 *recA*	0	0	$3{\cdot}4 \times 10^{-2}$

* Cam^R, chloramphenicol resistant phenotype; Kan^R, kanamycin resistant phenotype

Table 6. *Transduction experiments with 5K (rtl⁺ Kan^R) hybrids as donors*

Donor	Recipient	Marker selected*	Transductants per 10^7 phage	Unselected marker scored*	Number tested	Number sharing cotransduction
MW5651	AB1621	*xyl*⁺	8	—	—	—
		rtl⁺	2	Kan^R	200	200
				Cam^R	200	0
	KL144 *recA*	*aroC*⁺	0	—	—	—
		rtl⁺	0	—	—	—
		Kan^R	0·5	Cam^R	50	50
				rtl⁺	50	0
MW5652	AB1621	*xyl*⁺	9	—	—	—
		rtl⁺	3	Kan^R	50	50
				Cam^R	50	0
	KL144 *recA*	*aroC*⁺	0	—	—	—
		rtl⁺	0	—	—	—
		Kan^R	0·4	Cam^R	41	41
				rtl⁺	41	0

* Cam^R, chloramphenicol resistant phenotype; Kan^R, kanamycin resistant phenotype.

AB2575 and R4, which transferred different regions of the chromosome and, unlike 5K, were *gat*⁺. About 800 *rtl*⁺ colonies, 100 from each experiment, were tested. All were resistant to kanamycin and sensitive to the other antibiotics. They did not utilize galactitol, suggesting that the *rtl*⁺ genes had taken the usual chromosomal position at about 46 min. This was tested by conjugation, using the *rtl*⁺ Kan^R derivatives of Hfr strains AB2575 and R4 separately as donors with MLM161 (*his gat*⁺) as recipient, and selecting separately for Rtl⁺ and His⁺. The AB2575 derivative transferred *rtl*⁺ after 50 min and *his*⁺ after 48 min, and the R4 derivative transferred *rtl*⁺ after 62 min and *his*⁺ after 64 min, consistent with chromosomal locations for *rtl*⁺ and *his*⁺ near 46 min and 44 min, respectively. Of 2000 *rtl*⁺ transconjugants tested, all were resistant to kanamycin and none utilized galactitol.

It seemed that *rtl*⁺ genes and a kanamycin resistance determinant were located in the Hfr chromosome at about 46 min. However, *rtl*⁺ Kan^R need not have been in that position in the original hybrids, but might have been part of a plasmid derived from pE10 and capable of integration into the chromosome. If *rtl*⁺ was plasmid-borne in the four hybrids, then it should be transducible into *recA* bacteria. Alaeddinoglu (1976) showed pE10 to be transducible by P1kc into *recA* recipients, whilst chromosomal markers were not. Phage grown on hybrids MW5651 and MW5652 were used to transduce KL144 (*aroC recA*) and AB1621 (*xyl recA*⁺), selection being made for Rtl⁺, Aro⁺ and kanamycin resistance when KL144 was recipient, and for Rtl⁺ and Xyl⁺ when AB1621 was recipient (Table 6). AB1621 served as a positive control to show that *rtl*⁺ was transducible. Neither *aroC*⁺ nor *rtl*⁺ transductants of KL144 were obtained, whereas kanamycin resistant colonies occurred at low frequencies (one per 2×10^7 phage). Kanamycin resistant transductants did not utilize ribitol, but were resistant to chloramphenicol, neomycin,

tetracycline and ampicillin. Both *rtl*$^+$ and *xyl*$^+$ transductants of AB1621 were obtained with satisfactory frequencies. When tested, *rtl*$^+$ transductants were resistant to kanamycin but not to the other antibiotics and did not utilize galactitol. Judging from the evidence, the original hybrids contained two kanamycin resistance determinants, one linked to *rtl*$^+$ at about 46 min of the linkage map, and a second carried by pE10 or a derivative.

DISCUSSION

Crossing over in homologous regions flanking the *rtl atl* and *gat* regions would explain the mutually exclusive behaviour of the genes. Frequencies of transduction were comparable with frequencies for other genes, suggesting that the heterologous regions did not seriously interfere with crossing over and may be no more than a few genes long. Transductants were stable, giving no evidence of partial diploidy and pointing to considerable homology in the region.

Crossing over may have occurred in the heterologous region, but failed to be detected because of either the selections used or inviability of the recombinants. Incomplete exchange may give the observed results if smaller intrageneric regions conferred specificity on the enzymes or control systems.

An R plasmid proved useful for transferring *rtl*$^+$ from RK65 (*rtl*$^+$ Atl$^-$) to K12; *rtl*$^+$ mapped near *metG*. The complication that *rtl*$^+$ was 100% cotransducible with a kanamycin resistance determinant derived from the plasmid does not alter the previous findings. The extent of the affinity between *rtl*$^+$ and *kan*$^+$ is unclear. It may be that *kan* was a transposable element that became transposed during the mobilization of the *rtl*$^+$ genes, or was necessary for mobilization. Phage P1*clr100*KM carries a gene for kanamycin resistance, but there was no evidence that it became transposed during the transductions.

Differential segments of DNA may represent adaptation to different selective pressures (or, perhaps, different adaptations to the same selective pressure); such differences may prove useful in classifying strains as for *Salmonella* subtyping (e.g. Old, 1977). Similarities of gene order and function between *rtl*$^+$, *atl*$^+$ and *gat*$^+$ genes makes attractive the possibility of evolution from genes encoding less specific enzymes acting on polyols (Wu, 1976*a*, *b*). The 50% of strains which display none of the characters may possess other genes, or latent sequences or evolutionary remnants. If the regions immediately flanking the *rtl*$^+$ *atl*$^+$ and *gat*$^+$ genes are largely homologous in most wild strains, then the genes may have evolved by divergence in their position (Rigby *et al.*, 1974). If the genes are grouped with genes for other polymorphic characters, these groups would resemble the supergenes associated with polymorphism in higher organisms (Darlington & Mather, 1949).

Woodward & Charles (1982) introduced the concept of an ideal *E. coli* chromosome with characteristic positions for all genes, including luxury ones, most wild strains possessing only a proportion of luxury genes, or possessing only a proportion in an active form. They accepted that over larger chromosome distances, large blocks of genes were likely to be in different positions in different strains. The present experiments show that the concept must allow for different genes, or longer lengths of DNA, behaving as alternatives in the chromosome. Alaeddinoglu & Charles (1979) have already reported that genes for sucrose and D-serine utilization also behave as alternatives, but there is evidence that those genes may coexist in the chromosome in some strains (Hill, 1980).

The authors acknowledge the grant of an SRC studentship to M. J. Woodward.

REFERENCES

ADELBERG, E. A., MANDEL, M. & CHEN, G. C. C. (1965). Optimal conditions for mutagenesis by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in *Escherichia coli* K12. *Biochemical and Biophysical Research Communications* **18**, 788–795.

ALAEDDINOGLU, N. G. (1976). *Genetical analysis of variation in the ability to utilize sucrose in strains of Escherichia coli*. Ph.D. thesis, Reading University, U.K.

ALAEDDINOGLU, N. G. & CHARLES, H. P. (1979). Transfer of a gene for sucrose utilization into *Escherichia coli* K12, and consequent failure of expression of genes for D-serine utilization. *Journal of General Microbiology* **110**, 47–59.

BACHMANN, B. J. & LOW, K. B. (1980). Linkage map of *Escherichia coli* K12, edition 6. *Microbiological Reviews* **44**, 1–56.

BLUMENTHAL, T. (1972). P1 transduction: formation of heterogenotes upon cotransduction of bacterial genes with a P2 prophage. *Virology* **47**, 76–93.

CANNON, F. C., DIXON, R. A., POSTGATE, J. R. & PRIMROSE, S. B. (1974). Chromosomal integration of *Klebsiella* nitrogen fixation genes in *Escherichia coli*. *Journal of General Microbiology* **80**, 227–239.

CHARNETZKY, W. T. & MORTLOCK, R. P. (1974*a*). Ribitol catabolic pathway in *Klebsiella aerogenes*. *Journal of Bacteriology* **119**, 162–169.

CHARNETZKY, W. T. & MORTLOCK, R. P. (1974*b*). D-arabitol catabolic pathway in *Klebsiella aerogenes*. *Journal of Bacteriology* **119**, 170–175.

CHARNETZKY, W. T. & MORTLOCK, R. P. (1974*c*). Close genetic linkage of the determinants of the ribitol and D-arabitol pathways in *Klebsiella aerogenes*. *Journal of Bacteriology* **119**, 176–182.

CLARK, A. J. & MARGUILES, A. D. (1965). Isolation and characterization of recombination deficient mutants of *Escherichia coli* K12. *Proceedings of the National Academy of Sciences of the United States of America* **53**, 451–459.

COLSON, C., GLOVER, S. W., SYMONDS, N. & STACEY, N. A. (1965). The location of the genes for host controlled modification and restriction in *Escherichia coli* K12. *Genetics* **52**, 1043–1050.

COOPER, P. H., HIRSHFIELD, I. N. & MAAS, W. K. (1969). Map location of arginyl tRNA synthetase mutations in *Escherichia coli* K12. *Molecular and General Genetics* **104**, 383–390.

COWAN, S. T. & STEEL, K. J. (1974). *Manual for the identification of medical bacteria*, 2nd edn. Cambridge: Cambridge University Press.

DARLINGTON, C. D. & MATHER, K. (1949). *The elements of genetics*. London: Allen & Unwin.

EDWARDS, P. R. & EWING, W. H. (1972). *Identification of Enterobacteriaceae*, 3rd edn. Minneapolis: Burgess Publishing.

FERENCI, T. & KORNBERG, H. L. (1973). The utilization of fructose by *Escherichia coli*. Properties of a mutant defective in fructose-1-phosphate kinase. *Biochemical Journal* **132**, 341–347.

GOLDBERG, R. B., BENDER, R. A. & STREICHER, S. L. (1974). Direct selection for P1-sensitive mutants of enteric bacteria. *Journal of Bacteriology* **118**, 810–814.

HILL, S. H. A. (1980). *Genetic analysis of variation in Escherichia coli*. Ph.D. thesis, Reading University, U.K.

HILL, S. H. A. & CHARLES, H. P. (1980). Genetical analysis of natural variation in sucrose utilization in *Escherichia coli*. *Society for General Microbiology Quarterly* **7**, 82.

INDERLIED, C. B. & MORTLOCK, R. P. (1977). Growth of *Klebsiella aerogenes* on xylitol; implications for bacterial enzyme evolution. *Journal of Molecular Evolution* **9**, 181–190.

LENGELER, J. (1975*a*). Mutations affecting transport of hexitols D-mannitol, D-glucitol and galactitol. *Journal of Bacteriology* **124**, 26–38.

LENGELER, J. (1975*b*). Nature and properties of hexitol transport systems in *Escherichia coli*. *Journal of Bacteriology* **124**, 39–47.

LENGELER, J. (1977). Analysis of mutations affecting the dissimilation of galactitol in *Escherichia coli* K12. *Molecular and General Genetics* **152**, 83–91.

LENGELER, J. & STEINBERGER, H. (1978). Analysis of regulating mechanisms controlling the synthesis of the hexitol transport systems in *Escherichia coli*. *Molecular and General Genetics* **164**, 163–169.

LOW, B. (1973). Rapid mapping of conditional and auxotrophic mutations in *Escherichia coli* K12. *Journal of Bacteriology* **113**, 798–812.

MCCONVILLE, M. L. (1977). *A study of mutants of Escherichia coli defective in haem synthesis*. Ph.D. thesis, Reading University, U.K.

MCCONVILLE, M. L. & CHARLES, H. P. (1979). Isolation of haemin-requiring mutants of *Escherichia coli* K12. *Journal of General Microbiology* **113**, 155–164.

MORTLOCK, R. P., FOSSITT, D. D. & WOOD, W. A. (1965). A basis for utilization of unnatural pentoses and pentitols by *Aerobacter aerogenes*. *Proceedings of the National Academy of Sciences of the United States of America* **57**, 572–579.

OLD, D. C. (1977). The utilization of pentoses and pentitols by different biotypes of *Salmonella typhimurium*. *Journal of General Microbiology* **101**, 337–339.

PAULI, G. & OVERATH, P. (1972). The *ato* operon: a highly inducible system for acetoacetate and butyrate degradation in *Escherichia coli*. *European Journal of Biochemistry* **29**, 553–562.

PITTARD, J., LOUTIT, J. S. & ADELBERG, E. A. (1963). Gene transfer by F prime strains of *Escherichia coli* K12. I. Delay in initiation of chromosome transfer. *Journal of Bacteriology* **85**, 1394–1401.

REEVES, P. (1959). *Studies in bacterial genetics*. Ph.D. thesis, London University, U.K.

REINER, A. M. (1975). Genes for ribitol and D-arabitol catabolism in *Escherichia coli*: their loci in *Escherichia coli* C and absence in K12 and B strains. *Journal of Bacteriology* **123**, 530–536.

RIGBY, P. W. J., BURLEIGH, B. D. & HARTLEY, B. S. (1974). Gene duplication in experimental enzyme evolution. *Nature, London* **251**, 200–204.

RIGBY, P. W. J., GETHING, M. J. & HARTLEY, B. S. (1976). Construction of intergeneric hybrids using bacteriophage P1CM: transfer of the *Klebsiella aerogenes* ribitol dehydrogenase gene to *Escherichia coli*. *Journal of Bacteriology* **125**, 728–738.

SALISBURY, V., HEDGES, R. W. & DATTA, N. (1972). Two modes of curing transmissible bacterial plasmids. *Journal of General Microbiology* **70**, 443–452.

SCANGOS, G. A. & REINER, A. M. (1978). Ribitol and D-arabitol catabolism in *Escherichia coli*. *Journal of Bacteriology* **134**, 492–500.

TOMOEDA, M., INUZUKA, M., KUBO, N. & NAKAMURA, S. (1968). Effective elimination of drug resistance and sex factors in *Escherichia coli* by sodium dodecyl sulphate. *Journal of Bacteriology* **95**, 1078–1089.

WOODWARD, M. J. (1980). *Genetic analysis of variation in Escherichia coli*. Ph.D. thesis, Reading University, U.K.

WOODWARD, M. J. & CHARLES, H. P. (1980). Introduction of genes for sorbose utilization into *Escherichia coli* K12. *Society for General Microbiology Quarterly* **7**, 81.

WOODWARD, M. J. & CHARLES, H. P. (1982). Genes for L-sorbose utilization in *Escherichia coli*. *Journal of General Microbiology* **128**, 1969–1980.

WU, T. T. (1976*a*). Growth of a mutant of *Escherichia coli* K12 on xylitol by recruiting enzymes for D-xylose and L-1,2-propanediol metabolism. *Biochimica et biophysica acta* **428**, 656–663.

WU, T. T. (1976*b*). Growth on D-arabitol of a mutant strain of *Escherichia coli* K12 using a novel dehydrogenase and enzymes related to L-1,2-propanediol and D-xylose metabolism. *Journal of General Microbiology* **94**, 246–256.

WU, T. T., LIN, E. C. C. & TANAKA, S. (1968). Mutants of *Aerobacter aerogenes* capable of utilizing xylitol as a novel source of carbon. *Journal of Bacteriology* **96**, 447–456.