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Genetic Location of the Gene (*ush*) Specifying Periplasmic Uridine 5'-Diphosphate Glucose Hydrolase (5'-Nucleotidase) in *Escherichia coli* K-12

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Futher genetic mapping to two- and three-factor crosses show that the *ush* gene is closely linked to two other genes, *hemG* and *plsA*, and that the probable gene order is *proC-hemG-plsA-ush-gal*.

We have previously reported (2) the isolation and approximate map location of mutants that lack uridine 5'-diphosphate glucose hydrolase (*ush*$^-$), a periplasmic enzyme also known as 5'-nucleotidase. By interrupted-mating experiments the mutation mapped between *lac* and *gal*, in the region between 8 and 17 min on the *Escherichia coli* genetic map (1). In this report we describe more detailed genetic mapping. The strains used in this study are listed in Table 1. Linkage studies mediated by transduction crosses (Table 2) show that *ush* is unlinked (shows no co-transduction) to *proC* (min 9) but is linked to *purE* (min 12; 14% co-transduction) and is closely linked to *plsA* and *hemG* (min 11, see footnote to Table 1; 53 to 77% co-transduction). The linkages of *hemG*, *plsA*, and *ush* to each other are too similar to allow precise ordering. However, since *plsA* does not co-transduce with *proC* (6), whereas *hemG* does (H. P. Charles, personal communication), we assume that *hemG* lies to the left of *plsA* (Fig. 1). The co-transduction values of these loci to *purE* are consistent with this order and suggest that *ush* is between *plsA* and *purE* (Fig. 1).

To confirm the relative order of *plsA* and *ush* with respect to outside markers, three-factor crosses were performed by conjugation. Crosses 1 and 2 in Table 3 are two reciprocal crosses with either *ush*$^-$ or *plsA*$^-$ as the donor marker. The *proC* gene is distal to the *plsA* and *ush* genes, with respect to the origin of the Hfr (HfrC) used in these crosses, and the recipient is *proC*$^-$. Pro$^+$ recombinants were selected, and these were tested for segregation of the *plsA* and *ush* genes. In cross 1 (Table 3) the recombinant class Pls$^-$ Ush$^-$ should be the minority class, requiring four crossover events, if the order is *proC-plsA-ush*, whereas Pls$^+$ Ush$^+$ recombinants should be the minority if the order is *proC-ush-plsA*. The reverse situation applies to cross 2. The results of cross 1 clearly indicates that *ush* is to the right of *plsA*. Cross 2, however, is equivocal but may seem incompatible with cross 1, since the least frequent recombinant class is also Pls$^-$ Ush$^-$. A third cross was therefore performed, using a donor strain that transfers its chromosome in the opposite direction (HfrH) and selecting for the distal *gal*$^+$ gene, which is on the other side of *plsA-ush* (cross 3 in Table 2). The results clearly agree with cross 1, the minority recombinant class being that expected to result from four crossover events if the order is *plsA-ush-gal*.

We conclude from these crosses that the most compatible gene order is that depicted in Fig. 1. Concerning the unexpected high frequency of Pls$^+$ Ush$^+$ recombinants obtained in cross 2 (Table 2), it has been reported by others that in conjugal crosses where the donor has the Pls$^-$ phenotype (either *plsA*$^-$ or *plsB*$^-$) difficulties in obtaining certain types of recombinants are observed (5, 9). As noted recently by Levinthal (7), linkage relationships are conserved in closely related genera and may therefore reflect some selective advantage. In the case of the three genes reported here (*hemG*, *plsA*, *ush*), this advantage may possibly be related to the fact that at least two of the three genes and some other closely linked genes (*arc*, *min*, *lon*, *tsx*, *pho*, *brnQ*) have a function related to the cell surface.

We are grateful to those mentioned in Table 1 for their generous donation of strains. We particularly thank H. P. Charles and Malcolm McConville for their advice and for allowing us to quote their unpublished results.

TABLE 1. *List of bacterial strains*

Strain	Genotype[a]	Reference or source
5A-1	F⁻ *thr leu ara proA lac tsx gal his rpsL xyl mtl arg thi upp ush*	2
CSH57A	F⁻ *ara leu lacY proC gal purE trp his argG malA rpsL xyl mtl ilv metA* or *B thi*	8
CSH57A-12	F⁻ *ara leu lacY proC gal trp his argG malA rpsL xyl mtl ilv metA* or *B thi ush*	From 5A-1 × CSH57A by transduction and selection for Pur⁺
CSH57A-14	F⁻ *ara leu lacY proC gal trp his argG malA rpsL xyl mtl ilv metA* or *B thi plsA*	From CV2 × CSH57A by conjugation and selection for Pur⁺
CSH57A-14/43	F⁻ *ara leu proC gal trp his argG malA rpsL xyl mtl ilv metA* or *B thi ush plsA*	From CV2-4 × CSH57A-14 by conjugation and selection for Lac⁺
RLA6	F⁻ *lac proC phoR thi trp rpsL*	3
CSH62	HfrH *thi*	8
CV2	HfrC *plsA2 glpD3 glpR2 phoA8 tonA22* T2ʳ *rel-1*	J. Cronan (6)
CV2-4	HfrC *glpD3 glpR2 phoA8 tonA22* T2 *rel-1 ush*	From CV2 by transduction and selection for PlsA⁺
ABI62I[b]	*thi-1 hemA106 hemG47 mtl xyl ara gal lac tsx rpsL*	H. P. Charles
Puig 442[c]	*thi argH his pro thr hemG leu mtl xyl malA ara gal lacY tonA rpsL chlB*	H. P. Charles

[a] Genotype symbols are as in reference 1.

[b] This strain is a derivative of strain AB1621, which also carries a mutation allowing the uptake of hemin; the *hemG* gene is probably identical to that previously designated *popA* (M. McConville and H. P. Charles, personal communication).

[c] This strain is a derivative of strain Puig 442, which carries the *hemG47* mutation from AB1621 plus a second mutation in the *hemG* gene that allows aerobic growth and results in accumulation of a hemin precursor. Colonies carrying this *hemG* allele are therefore brown and resemble the *popA* mutants (M. McConville and H. P. Charles, personal communication; 4).

TABLE 2. *Two-factor crosses by transduction*[a]

Donor and relevant genotype	Recipient and relevant genotype	Selected phenotype	No. of colonies tested	No. (%) of colonies of unselected donor phenotype		
				Ush	Pls	Hem
5A-1 *ush*⁻	RLA6 *proC*⁻	Pro⁺	319	0		
5A-1 *ush*⁻	CV2 *plsA*⁻	Pls⁺	156	113 (72.5)		
5A-1 *ush*⁻	CSH57A *purE*⁻	Pur⁺	307	43 (14)		
5A-1 *ush*⁻	AB1621 *hemG*⁻	Hem⁺	159	123 (77)		
CV2 *plsA*⁻	AB1621 *hemG*⁻	Hem⁺	84		76 (91)	
CSH57A-14/43 *ush*⁻ *plsA*⁻	AB1621 *hemG*⁻	Hem⁺	159	99 (62.2)	115 (72.3)	
Puig 442 *hemG*⁻	CSH57A-14/43 *ush*⁻ *plsA*⁻	Pls⁺	134	71 (53)		86 (64)

[a] Recipient cells were either exponential-phase cells in R broth (containing, per liter: 10 g of tryptone, 1 g of yeast extract, 1 g of glucose, and 5 g of sodium chloride) or overnight cells grown in TS medium (per liter: 3 g of Soyatone, 5 g of sodium chloride, 17 g of tryptone, and 2.5 g of dipotassium phosphate). Both media gave comparable transduction frequencies. For transduction phage, P1*vir* was used at a multiplicity of approximately 0.2. The phage was allowed to adsorb to the cells in the presence of 5 mM $CaCl_2$ for 20 min at 37°C. After this time the cells were centrifuged, resuspended in phosphate buffer (pH 7.0) containing 0.2% sodium citrate, and plated on selective media containing M9 buffer (8), 0.2% sodium citrate, and, where required, amino acids, adenine and hemin at 20 μg/ml, and thiamine and δ-aminolevulinic acid at 10 μg/ml. The *hemA*⁻ mutation in strain AB1621 is satisfied by δ-aminolevulinic acid or hemin, whereas the *hemG*⁻ mutation is only satisfied by hemin (H. P. Charles, personal communication). Accordingly, for selection of HemG⁺ recombinants the plates contained δ-aminolevulinic acid. The HemG⁻ phenotype in the cross involving strain Puig 442 was scored by noting the color of purified recombinants on R broth media; HemG⁻ recombinants are brown, whereas HemG⁺ recombinants are white (see 4 and Table 1). The Ush phenotype was scored on plates containing adenosine 5′-monophosphate (3 mM) as carbon source; the presence of small amounts of amino acids, such as those required as supplements for CSH57A, seemed to improve growth on adenosine 5′-monophosphate, presumably by allowing some background growth. A sample of Ush⁺ and Ush⁻ recombinants from many of the crosses was checked by direct assay (2) for the presence or absence of 5′-nucleotidase.

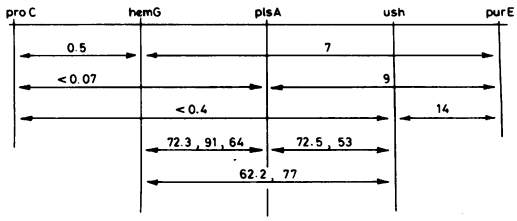

FIG. 1. *Summary of transduction data. Values are given as percentage of co-transduction. Data on the two upper horizontal lines are from M. McConville and H. P. Charles (personal communication) or Cronan and Godson (6).*

TABLE 3. *Analysis of recombinants from crosses by conjugation*[a]

Cross	Donor and relevant genotype	Recipient and relevant genotype	Selected phenotype	No. of colonies tested	% Colonies of unselected phenotype:
1	CV2-4 HfrC *ush*	CSH57A-14 F⁻ *proC plsA strA*	Pro⁺ Str^r	72	Pls⁺ Ush⁻, 79 Pls⁺ Ush⁺, 8.4 Pls⁻ Ush⁺, 12.4 Pls⁻ Ush⁻, 0
2	CV2 HfrC *plsA*	CSH57A-12 F⁻ *proC ush strA*	Pro⁺ Str^r	148	Pls⁺ Ush⁻, 40.7 Pls⁺ Ush⁺, 6.9 Pls⁻ Ush⁺, 49.7 Pls⁻ Ush⁻, 2.8
3	CSH62 HfrH	CSH57A-14/43 *plsA ush gal strA*	Gal⁺ Str^r	129	Pls⁺ Ush⁻, 0.8 Pls⁺ Ush⁺, 51.5 Pls⁻ Ush⁺, 4.0 Pls⁻ Ush⁻, 43.7

[a] Exponential-phase Hfr and F⁻ cells, grown in R broth, were mixed in a ratio of 1:10 and left for about 60 min at 25 or 29°C. The cells were then diluted and plated on the appropriate minimal selective plates (see footnote to Table 2); streptomycin was added at 100 μg/ml. Str^r, Streptomycin resistance.

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