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Chromosomal Location of the Gene Determining Uridine Diphosphoglucose Formation in *Escherichia coli* K-12

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Strains of *Escherichia coli* K-12 which cannot synthesize uridine diphosphoglucose (UDPG) have a complex phenotype. They are host-modifying for the T-even phages (A. Shedlovsky and S. Brenner, Proc. Natl. Acad. Sci. U.S. **50**:300, 1963; S. Hattman and T. Fukasawa, Proc. Natl. Acad. Sci. U.S. **50**:297, 1963). They cannot ferment galactose (T. Fukasawa, K. Jokura, and K. Kurahashi, Biochim. Biophys. Acta **74**:608, 1963) and are sensitive to galactose-induced bacteriostasis in minimal salts-glycerol medium (T. Sundrarajan, A. Rapin, and H. Kalckar, Proc. Natl. Acad. Sci. U.S. **48**:2187, 1962). They are sensitive to phage C21 because of their inability to incorporate galactose into the cell wall (A. Shedlovsky and S. Brenner, Proc. Natl. Acad. Sci.

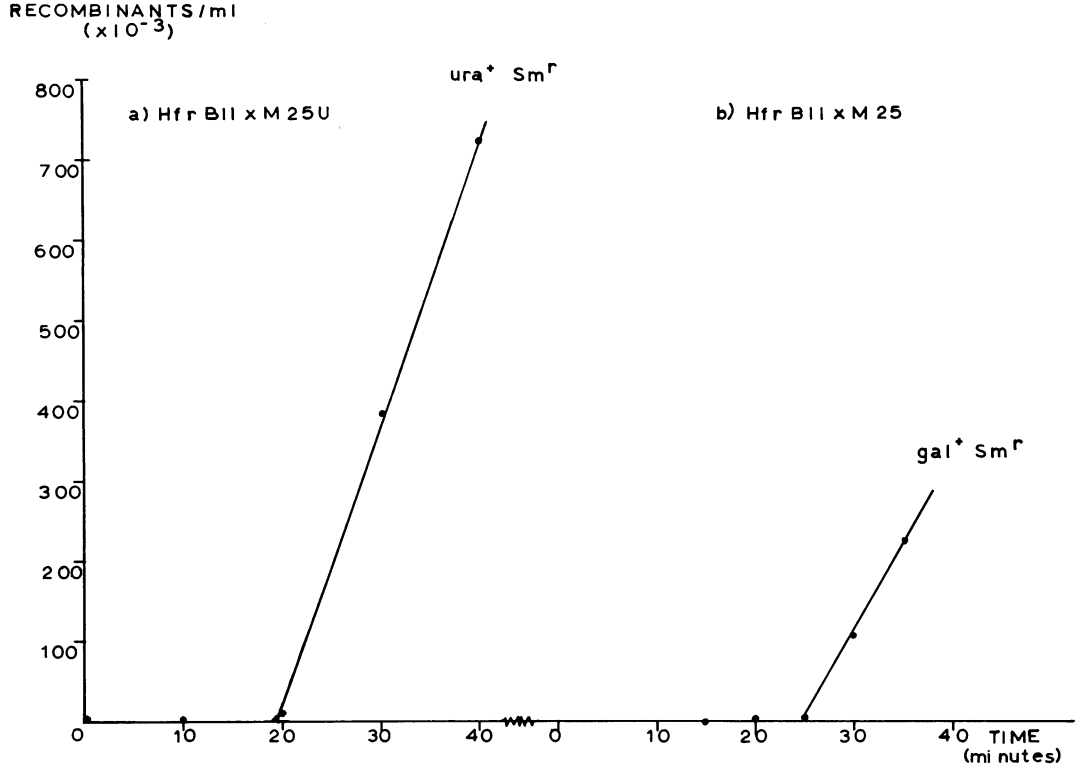

FIG. 1. *Crosses between strain B11 and strains M25 and M25U. Parent cultures were grown without aeration to approximately 10^8 per milliliter in peptone (1%)-glucose (0.5%) medium buffered with K_2HPO_4 (0.2%); (20 μg/ml of uracil was added when necessary. At time 0, 1-ml samples of each parent were put in replicate tubes, and these tubes were then incubated at 37 C without aeration. At the time indicated on the abscissa, a 0.1-ml sample was taken from one tube and diluted into 9.9 ml of cold buffer, agitated on a vortex mixer for 90 sec, and plated on either minimal salts-glucose medium (a) or eosin-methylene blue-galactose medium (b) containing 125 μg of streptomycin per ml.*

U.S. **50**:300, 1963). The gene for the enzyme which catalyzes the formation of UDPG, UDPG pyrophosphorylase, lies outside the *gal* region of the chromosome, since it is not carried by λdg (T. Fukasawa, K. Jokura, and K. Kurahashi, Biochem. Biophys. Res. Commun. **7**:121, 1962) or F-*gal*$^+$ (H. Echols, J. Reznichek, and S. Adhya, Proc. Natl. Acad. Sci. U.S. **50**:286, 1963).

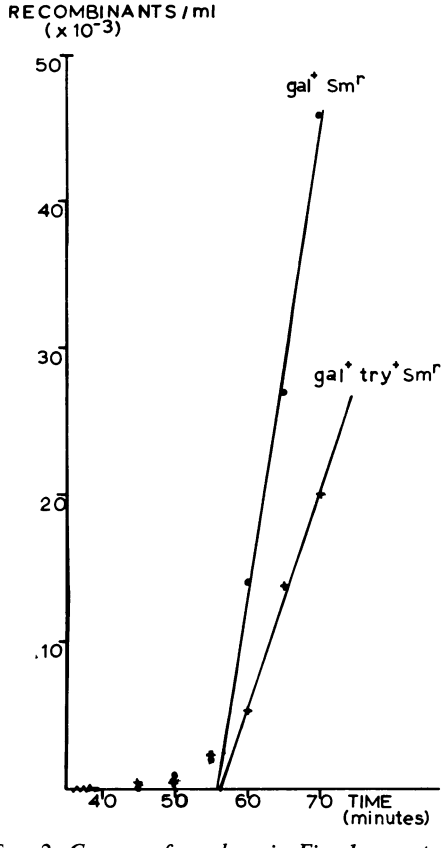

FIG. 2. *Cross performed as in Fig. 1 except that the tubes were incubated at 32 C. The crosses indicate gal*$^+$ *colonies appearing on the eosin-methylene blue-galactose-Casamino Acids medium, and the solid circles indicate gal*$^+$ *colonies appearing on the eosin-methylene blue-galactose-peptone medium.*

Two *UDPG*$^-$ alleles, designated gal^-_{U95} and gal^-_{U106}, both isolated in Cambridge by S. Brenner, were used to determine the location of the UDPG pyrophosphorylase gene; gal^-_{U95} has been shown to lack UDPG pyrophosphorylase activity (Fukasawa, *personal communication*), and gal^-_{U106} is thought to carry a defect in the same gene. An allelism test has shown a recombination frequency between the two markers of less than 5×10^{-3}. Moreover, gal^-_{U106} is not a phosphoglucomutase mutant since its sensitivity to phage C21 is not corrected by growth on maltose.

Interrupted-mating experiments with Hfr B11 (W. Hayes, *The Genetics of Bacteria and Their Viruses*, p. 568, Blackwell, Oxford, 1964) and the nearly isogenic strains M25 (F$^-$ gal^-_{U95} *str-r*) and M25U (F$^-$ *ura pyrD str-r*) showed that the *UDPG* gene is located close to the tryptophan region of the K-12 chromosome; at 37 C, *gal*$^+$ *str-r* recombinants began to appear approximately 5 min later than *ura*$^+$ *str-r* recombinants (Fig. 1). To find the position of the *UDPG* locus relative to the tryptophan operon, an interrupted-mating experiment was performed at 32 C with Hfr B11 and strain M226 (F$^-$ gal^-_{U106} *try str-r*). Samples were plated on eosin-methylene blue-galactose medium containing streptomycin and either 1% peptone or 1% Vitamin Free Casamino Acids (Difco) as nutrient base. On the Casamino Acids medium, only *try*$^+$ recombinant cells can give rise to black *gal*$^+$ colonies, whereas on the peptone medium both *try* and *try*$^+$ cells grow into black *gal*$^+$ colonies. Hence, only if the tryptophan gene enters the F$^-$ cell first will the plots for the appearance of *gal*$^+$ colonies on the two media be roughly similar. As Fig. 2 shows, the *UDPG* gene enters the F$^-$ cell slightly before the *try* gene. (The difference in slope of the two plots is too large to be accounted for by recombination between the two loci, which is approximately 15%).

Final confirmation of this result came from a four-factor cross utilizing the *purB* (*ade*), *try*, and *pyrF* (*ura*) markers, which have been located in a small region of the K-12 chromosome (E. Sig-

TABLE 1. *HfrH* gal^-_{U106} × F$^-$ *ade* (*purB*) *try ura* (*pyrF*) *str-r*[a]

No. of crossovers.....	0	1			2			3
Recombinant genotype.....	ABCD	abcD	abCD	aBCD	ABcD	AbcD	AbCD	aBcD
No. of recombinants.....	353	23	24	79	2	9	59	0
	341	26	41	88	4	9	45	0

[a] Crosses were performed as in Fig. 1 except that the growth medium was Penassay Broth (Oxoid). The mating mixture was incubated at 37 C for 2.5 hr before plating; *ura*$^+$ *str-r* recombinants were selected on appropriately supplemented minimal medium, stabbed into the selective medium, and replica-plated to analyze the inheritance of unselected markers: A, +; B, U106; C, +; D, +; a, *purB*; b, +; c, *try*; d, *pyrF*.

ner, J. Beckwith, and S. Brenner, J. Mol. Biol. **14**:153, 1965). The results of duplicate experiments are shown in Table 1. The order of the markers is clearly *purB—UDPG—try—pyrF*. The distortion of one doubly recombinant class is due to failure of the recombinants to segregate before plating, since the distorted class has the dominant allele at each of the three loci. (Crosses performed under the same conditions regularly show approximately 5% heterogeneous colonies for non-selected fermentation markers when plated directly on eosin-methylene blue medium.) In a subsequent cross of Hfr B11 *try* with an F^- *ade* (*purB*) gal^-_{U106} *pro str-r* strain, all of the pro^+ try^+ *ade str-r* recombinants tested carried the $UDPG^-$ marker.

Phages $\phi 80$ and $\lambda c_{I857} h80$ (Signer, J. Mol. Biol. **15**:243, 1966) transduce both gal^-_{U106} and gal^-_{U95}. H. Echols (*personal communication*) has independently isolated a $\phi 80\delta_{dUDPG}$ and found that the $\phi 80$ dsu_c^+ of Signer also carries the *UDPG* locus. This places the *UDPG* gene on the *purB* side of *att80*. Moreover, the *UDPG* gene lies between su_c^+ and *att80*, because all $\phi 80$ dsu_c^+ phage carry the *UDPG* marker, and there are transducing $\phi 80$ which carry the *UDPG* locus but not the suppressor (S. Brenner, *personal communication*).

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