JOURNAL OF BACTERIOLOGY, July 1976, p. 406-417

Vol. 127, No. 1
Printed in U.S.A.

Regulation of β-Glucuronidase Synthesis in *Escherichia coli* K-12: Constitutive Mutants Specifically Derepressed for *uidA* Expression

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Received for publication 14 April 1976

All methyl-β-D-galacturonide-positive mutants isolated from *Escherichia coli* K-12 carry constitutive mutations for β-glucuronidase (UID) synthesis. Most of these mutants are specific for UID synthesis and are distributed in three classes according to the derepression level of UID. Each specific mutant carries a mutation(s) near *uidA*, the structural gene for UID, at min 30.5 of the *E. coli* K-12 linkage map. The expression of UID synthesis in F-merodiploid strains carrying these mutations permits discrimination between dominant and recessive constitutivity over the wild-type allele. The first kind of mutation (dominant) should affect the operator site *uidO* of the structural gene *uidA*; the second type of mutation (recessive) should affect a regulatory gene, *uidR*, operating through a negative control. The isolation of mutants bearing at this locus superrepressed mutations, which can revert to produce a constitutive phenotype, confirms the occurrence of such a regulatory gene. The partially derepressed *uidR* mutants of the first class are normally inducible and remain constitutive at low temperature; their UID has the same thermal sensitivity as in the wild-type strains. The occurrence of similar regulatory gene mutants has been recently described in the lactose system (Shineberg, 1974).

β-Glucuronidase (UID) is the first enzyme of the hexuronide-hexuronate pathway in *Escherichia coli* (Fig. 1). Several observations indicate that the regulation of UID synthesis is independent from that of the other enzymes of the pathway (1). First, it does not exhibit the same type of induction (1, 9); moreover, superrepressed or constitutive mutants have been isolated in which only the synthesis of glucuronate and of galacturonate enzymes has been affected, whereas that of UID remains inducible (21).

Although β-galacturonides are substrates of UID, they cannot induce this enzyme as do β-glucuronides. The former cannot, alone, be used as a carbon source for the wild-type strain of *E. coli* (3, 4). Methyl-β-galacturonide (MeGalU), therefore, has been used as a noninductive substrate to select regulatory mutants constitutive for UID synthesis (3, 4).

We found two different types of MeGalU$^+$ mutants: (i) specific mutants, derepressed only for UID synthesis; (ii) pleiotropic mutants, derepressed for both UID and mannonate oxidoreductase (MOR).

The present work deals with the isolation and characterization of MeGalU$^+$ mutants and with the behavior and genetic mapping of mutations carried by the specific mutants only.

In the accompanying article (13) we examine more particularly the pleiotropic mutants and discuss the regulation of UID synthesis in general. A short note has already been published on this work (12).

MATERIALS AND METHODS

Nomenclature. The genetic nomenclature is according to Taylor and Trotter (22). A new symbol, *uidA*, is used instead of *gurA* for the structural gene for UID (10). Gur$^+$ or Uid$^+$ indicates growth on methyl-β-D-glucuronide (MeGlcU). We propose *uidR* as the specific regulatory gene for the *uidA* expression and *uidO* as the operator site of the *uidA* gene.

Bacterial strains. All of the bacterial strains used were *E. coli* K-12 derivatives; they are listed in Table 1.

Media. M63 mineral medium (19) was supplemented as described elsewhere (10). MeGalU was added to a final concentration of 2 mg/ml. Other media were described earlier (10).

Current genetic methods. Conjugation with Hfr strains and transduction with phage P1*kc* were performed as described previously (10).

Mutagenesis. Independent clones were isolated, their properties were examined, and the clones were then grown overnight on mineral medium with glycerol.

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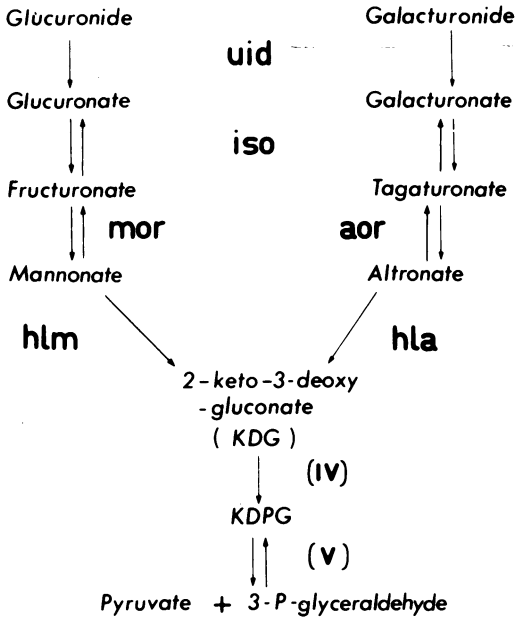

FIG. 1. *Metabolic pathway of degradation of hexuronides and hexuronates in E. coli. UID, β-Glucuronidase (EC 3.2.1.31); ISO, uronate isomerase (EC 5.3.1.12); MOR, mannonate oxidoreductase (EC 1.1.1.57); HLM, mannonate hydrolyase (EC 4.2.1.8); AOR, altronate oxidoreductase (EC 1.1.1.58); HLA, altronate hydrolyase (EC 4.2.1.7); IV, 2-keto-3-deoxygluconate (KDG) kinase (EC 2.7.1.45); V, 2-keto-3-deoxygluconate-6-phosphate (KDPG) aldolase (EC 4.2.1.14).*

EMS treatment. Overnight cultures were diluted in mineral medium and grown for two to three generations. Cells in exponential phase (3×10^8 to 4×10^8 cells per ml) were centrifuged and suspended in phosphate buffer (20 mM, pH 7.1) (9×10^8 cells per ml). After addition of 0.24 M ethyl methane sulfonate (EMS), the cell suspension was shaken for 30 min at 37 C, and the reaction was stopped by dilution in 10 volumes of M63 medium.

UV treatment. Exponential-phase cultures were centrifuged, diluted in phosphate buffer (2×10^8 cells per ml), and irradiated in a petri dish under an ultraviolet (UV) germicidal lamp (Mineralight) under conditions such that 1 of 10^3 cells survived.

NTG treatment. Overnight cultures (1.3×10^9 cells per ml) were centrifuged and suspended in an equal volume of acetate buffer (0.1 M, pH 4.6). *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG; 100 μg/ml) was added, and the cell suspension was incubated for 30 min without shaking at 37 C. The reaction was stopped by dilution in 5 volumes of L-broth (10).

Sodium nitrite (NO_2) treatment. Overnight cultures were centrifuged, washed, and diluted in acetate buffer (0.1 M, pH 4.6) (6×10^8 cells per ml) with 1.5 mM NO_2. After incubation for 25 min without shaking at 37 C, the reaction was stopped by addition of an equal volume of tris(hydroxymethyl)-aminomethane-hydrochloride (1 M, pH 8.0).

Isolation of MeGalU-positive mutants. Mutagenized or nontreated cells were centrifuged and diluted sixfold in M63 medium with glycerol. After overnight growth to allow phenotypic expression of presumed mutations, mutagenized cultures were centrifuged and suspended in mineral medium. All of the cell suspensions were diluted (1.5×10^8 cells per ml) in M63 medium with MeGalU as the sole carbon source and aerated at 37 C for 5 to 6 days. Agitation was then stopped, but the incubation was continued for 10 to 14 days.

Characterization of the constitutive UID mutants. When visible growth appeared in liquid cultures, the samples were diluted and spread on L-agar medium. Isolated colonies were replicated on various media to examine: (i) their Hfr character; (ii) their auxotrophy, when necessary; and (iii) the nature of UID synthesis in these clones. For this test, replicates were made on glycerol-agar medium. After overnight growth, a few drops of a solution of 10 mM *p*-nitrophenyl-β-D-glucuronide (pNPGlcU) and chloramphenicol (50 μg/ml) were placed on the surface of the agar plate. Yellow-colored colonies indicated the occurrence of UID synthesis in the absence of any inducer. pNPGlcU-positive clones were isolated and examined once again. Finally, each presumptive mutant strain was assayed for UID activity after growth in liquid cultures on glycerol; cells were harvested by centrifugation during the exponential phase, suspended in phosphate buffer (20 mM, pH 7.1) (0.1 to 0.8 mg of bacterial dry weight), and shaken with toluene (1:35 to 1:50, vol/vol) at 37 C for 30 min (9).

UID assay. The UID assay was carried out in the same phosphate buffer with 2-mercaptoethanol (0.1 M) and pNPGlcU (1 mM) at 30 C. The reaction was initiated by the addition of a diluted enzyme preparation, and the absorbance of the *p*-nitrophenol liberated was recorded at 405 nm for 5 to 10 min in a Unicam spectrophotometer recorder (SP 800) or a Jobin et Yvon Ultraspac recorder. One unit of UID activity is defined as the amount of enzyme hydrolyzing 1 μmol of pNPGlcU per min under the above-mentioned conditions. Specific activity is expressed as UID units per milligram of bacterial dry weight.

Dye test for MOR activity. Dye tests were performed on clones grown overnight on glycerol-agar medium (14). Only clones derepressed for MOR activity show a color reaction in the presence of mannonate and tetrazolium salt under the defined conditions.

Induction of UID activity. Overnight glycerol cultures were diluted in the same medium supplemented with inducer(s) at the concentration indicated (9). Cultures were stopped after three to four generations, and the cells were washed and treated as described above.

Preparation of an F-prime strain covering the *uidA* region. As we already noted (10), neither of the F-prime strains, F148 and F123, obtained from B. Low, covers the *uidA,man-4* region. Using the Low method (7), we succeeded in isolating an F-prime strain (F500) spanning the *his,aroD,uidA,man-4*

TABLE 1. *Bacterial strains*

Strain	Sex	Genotype[a]	Origin or derivation
HfrH 3000	Hfr	*thi-1*	Pasteur Institute (Paris)
HfrH 3300	Hfr	*thi-1,lacI22*	E. Wollman (via J. Puig)
P4X	Hfr	*metB1*	E. Wollman (via J. Puig)
KL16	Hfr	*thi-1*	Pasteur Institute (Paris)
KL16-99	Hfr	*recA1*	K. B. Low (via J. Puig)
KL96	Hfr	*thi-1*	K. B. Low (via B. Bachmann)
JB1	Hfr	*metB1,uxuA1*	From P4X (16)
CM1	Hfr	*metB1,uxuB1*	From P4X (16)
CM8	Hfr	*metB1,uxuB8*	From P4X (15)
AU128	Hfr	*metB1,uxaC*	From P4X (9)
PA309	F⁻	*thr-1,leu-6,thi-1,his-1,argH1,trp-1,str-9,lacY1,malA1,xyl-7,mtl-2,gal-6,ara-13*	E. Wollman (via J. Puig)
PA601	F⁻	Like PA309 but *trp⁺,purE43,proC*	E. Wollman (via J. Puig)
K63	F⁻	*his,fadD88,man-4,gal,str*	P. Overath (via J. Pouyssegur)
GM146	F⁻	*his-4,argE3,aroD6,thi-1,str-700,galK2,lacY1,xyl-5,mtl-1*	Gur⁺ derivative of AB3302 (from J. Pittard)
GM291	Hfr	*metB1,uidA1*	From P4X (10)
GM290	F⁻	*argE3,aroD6,uidA1,str-700,galK2,lacY1,xyl-5,mtl-1*	From GM146 by conjugation with GM291 (10)
GMS343	F⁻	*argE3,aroD6,str-700,mtl-1,man-4,lacY1,galK2*	From GM290 by P1 transduction from K63
GMS529	F⁻	Like GMS343 but *thy*	Trimethoprim resistance (20)
GMS724	F⁻	Like GMS529 but *metB,recA,thy⁺,arg⁺*	*thy⁺* derivative from a cross between GMS529 and KL16-99 after *arg⁺,met* P1 transduction from P4X
GMS407	F⁻	Like GMS343 but *uidA1,aro⁺,str⁺*	(10)
GMS838	Hfr	*argE3,man-4*	Gur⁺ *man* derivative from GM291 by P1 transduction from K63 and then *met⁺,argE* by P1 transduction from GM146
F500/S724	F′	F *aroD⁺,man⁺*/S724	This study
F500/KL159	F′	F *aroD⁺,man⁺/thi-1,his-4,aroD5,xyl-5,proA2,recA1*	This study; KL159 from B. Low (via B. Bachmann)
GM241	F⁻	Like PA601 but *uidA2*	NTG mutant of PA601 (10)
GMN81	F⁻	Like PA601 but *uidR101*	NTG mutant of PA601 (11)
GMN121	F⁻	Like PA601 but *uidR102*	NTG mutant of PA601 (11)
GRS182	F⁻	Like GMN81 but *uid⁺*	Uid⁺ spontaneous revertant from GMN81

[a] The allele numbers of usual markers are those of the Coli Genetic Stock Center, Yale University.

region (see Fig. 3). This was done by crossing the Hfr strain KL96 with *recA,man* F⁻ recipients. Man⁺ exconjugants were isolated and examined by replica platings for their ability to transfer the wild-type phenotypes (Aro⁺,Uid⁺,Man⁺) to suitable F-minus strains.

Two kinds of conjugants appeared. The first consisted of good donors that had become Rec⁺ (UV resistant). Type II episome formation (8) probably occurred in these strains. The *recA⁺* gene is a distal marker for this Hfr and is therefore located near the F factor; this gene may be included in the episomes formed, leading to the integration of the F factor. The second kind, the majority, remained *recA* and donors of His⁺, Aro⁺, Uid⁺, and Man⁺ but not of Gal⁺ or Thy⁺, a property that is consistent with a type I episome formation (8).

Only the strains exhibiting a stable donor character were retained for genetic and physiological experiments.

Episomes selected in this region gave strongly mucoid cultures. Mucoidness was attributed to the diploid state of the *uvrC* gene (8) at min 36 of the chromosome; it greatly hinders practical use of these strains because of difficulties in their isolation and slow growth, but up to now a convenient, stable, nonmucoid episome has not been found. Segregation of these episomes was prevented by maintaining them constantly on Aro⁺-selective medium.

Analysis of UID synthesis in merodiploid strains. Crosses were performed between wild-type (pNPGlcU⁻, Uid⁺) F-prime strains and F-minus (*recA*) strains carrying independent mutations. Conjugants Aro⁺ and Man⁺ were isolated and characterized. Liquid cultures containing glycerol (or eventually mannose) as the sole carbon source were

undertaken immediately, and samples from these cultures were assayed for UID activity, as described above. To measure the apparent segregation rate in each culture, samples were diluted, spread on L-agar medium, and replicated overnight on suitable media.

Chemicals. MeGlcU (F. Stoeber, D.Sc. thesis, Univ. of Paris, Paris, France, 1961), MeGalU (3), and mannonic amide (16) were synthesized in this laboratory. D-Glucuronate was purchased from Sigma Chemical Co.; pNPGlcU was from Sigma Chemical Co., Calbiochem Co., or Boehringer Mannheim; NTG was from Aldrich Chemical Co. or Fluka AG. Chem. Fab.; EMS was from Koch-Light; and trimethoprim was from Calbiochem.

RESULTS

Isolation and characterization of MeGalU$^+$ mutants. Independent clones from wild-type Hfr strains, HfrH, KL16, and P4X and from P4X Uxu$^-$ mutants were grown with MeGalU as the sole carbon source, without any prior treatment or after NTG, EMS, NO_2, or UV treatment. After a long incubation period (5 to 6 and sometimes 10 to 20 days) at 37 C, growth appeared, and we retained one clone from each culture, except when clear differences of activity were observed. We thus isolated a total of 41 clones considered to be independent mutants (Table 2).

All of the MeGalU$^+$ mutants were screened for constitutive UID or MOR synthesis by the assay on solid medium described above. After purification, UID activity was measured in noninduced cultures in the middle of the exponential phase. A direct analysis of MOR derepression was not possible with the Uxu$^-$ (MOR$^-$) mutants. With the Uxu$^+$ strains, we found that 8 of 32 were derepressed for both enzymes and 24 of 32 were derepressed only for UID synthesis.

Apparently there is no correlation between the mutagenic treatment and the appearance of mutants belonging to one of these two classes (Table 2). Moreover, we observed from the same initial clone and after the same treatment the appearance of different types of mutants, as shown by the level of their UID or MOR activity (e.g., GMX21 and GMX23, from clone 5, in Table 2). The constitutive level of UID was quite variable among these mutants, and their activity (Fig. 2) can be divided into three classes. The major one consists of specific UID mutants derived from all of the strains and of pleiotropic mutants derived from strains KL16 and HfrH (class II). Its activities vary between 3 and 8 U/mg, which corresponds to a derepression rate of 600- to 1,600-fold that of the basal level or 0.33- to 1-fold that of the induced level of the corresponding wild-type strain (9). The pleiotropic mutants derived from strains P4X and HfrH form a group (class III) with activities from 11 to 15 U/mg. In this case, the derepression rate was 2,000- to 3,000-fold that of the basal level (or 1.5- to 2-fold that of the induced level). Finally, two UID-specific mutants stand out because of their weak constitutive activity, less than 1 U/mg, and correspond to class I. Nevertheless, these activities are still some 100- to 200-fold higher than the basal level of the wild type.

Genetic mapping of the constitutive mutations specific for UID synthesis. A mutant derepressed for UID synthesis only, GMK1 (Hfr KL16), was crossed, by uninterrupted mating, with strain PA309 (F$^-$ *his,trp,leu,thr,arg,str*). Recombinants that were Str^R and either His$^+$,Trp$^+$ or Thr$^+$,Leu$^+$ were selected. The constitutive pNPGlcU-positive character of the Hfr strain was preferentially inherited by the Trp$^+$ (Str^R) recombinants. This phenotype was expressed in the recombinants with the same intensity as in the Hfr strain, except for certain cases that we were able to relate to the presence of some auxotrophic markers (see below).

After multiplication of phage P1 on this GMK1 mutant and transduction to appropriate recipient strains, the locus responsible for pNPGlcU-positive phenotype appeared to be strongly cotransducible with *man*$^+$ (94%) and weakly with *aroD*$^+$ (4%) (Table 4).

Analysis by transduction of all the other specific mutants yielded similar results (Tables 3 and 4). This allows the location of a constitutive determinant at min 30.5, close to *uidA* structural gene for UID. The very close proximity of these two loci is confirmed by the very low frequency of crossing-over resulting in an inducible phenotype (Fig. 3; Table 4).

Determination of the monogenic character of the constitutive mutations. In certain recombinants resulting from crosses of F$^-$-polyauxotrophic strains, the UID activity is decreased compared with the activity of the mutant Hfr strain, e.g., GMS474 and GMK1 (Table 5).

The decrease could result from the dissociation of two gene determinants, constitutively different. This hypothesis, however, has been discarded since the original activity of GMK1 was mostly recovered by simple transduction of the allele *trp*$^+$ upon the GMS474 strain (see strain GMS507, Table 5). The repressing effect in auxotrophic strains is not yet well understood (G. Novel, D.Sc. thesis, Univ. of Lyon, Lyon, France, 1973).

To limit the variations of UID activity due to this parasitic effect we chose, as a recipient of the constitutive mutations, derivatives of

TABLE 2. *MeGalU-positive mutants of E. coli K-12: origin and enzymatic characteristics*

Original strain	Clone no.	Origin of mutation	Mutant strain	UID-constitutive activity: rank no. (Fig. 2)	MOR⁻ constitutivity test
HfrH (3000)		[a]	GM230	II	+
HfrH (3300)	2	NTG	GMH34	II	+
		EMS	GMH43	II	−
		NTG[b]	GMH47	II	+
	1	UV	GMH42	II	−
		UV	GMH39	III	−
	3	Sp[c]	GMH51	II	−
	6	Sp	GMH35	II	−
KL16		[a]	GMK1	II	−
		[a]	GMK2	II	+
		[a]	GMK3	II	−
		[a]	GMK4	II	−
		[a]	GMK5	II	−
P4X	1	Sp	GMX29	I	−
		EMS	GMX19	II	−
	2	Sp	GMX13	II	−
		EMS	GMX1	III	−
		NO_2[d]	GMX24	II	−
	5	UV	GMX21	III	+
		UV	GMX23	II	−
	3	Sp	GMX37	II	−
		NTG	GMX10	III	+
		NTG	GMX11	II	−
	4	NTG	GMX5	III	−
	9	Sp	GMX34	II	−
	10	Sp	GMX31	I	−
	6	UV	GMX27	II	−
JB1	1	Sp	GMX53	II	−
	2	Sp	GMX58	III	+
		Sp	GMX59	II	−
	3	Sp	GMX48	II	−
	4	Sp	GMX49	III	+
CM1	2	Sp	GMX47	II	[e]
	4	Sp	GMX51	II	[e]
	5	Sp	GMX52	II	[e]
	1	Sp	GMX56	II	[e]
	3	Sp	GMX57	II	[e]
CM8	1	Sp	GMX54	II	[e]
	3	Sp	GMX46	II	[e]
	4	Sp	GMX50	II	[e]
	5	Sp	GMX55	III	[e]

[a] M. L. Didier-Fichet; origin not certain.
[b] Independently obtained on MeGalU agar in the presence of NTG.
[c] Sp, Spontaneous.
[d] NO_2, Sodium nitrite.
[e] Because of the MOR-negative mutation carried by these strains, the color test for this enzyme is not required.

strain AB3302, which is but seldom affected by this inhibitory effect (GMS343 and GMS529) and we used P1 transduction to transfer these mutations, so as to limit maximally the heterogeneity of the chromosome.

Under these conditions of transduction, we observed that the UID activity for mutations of type I or II remained stable, strongly suggesting a monogenic character.

The hyperderepressed mutants of type III, however, appear to be more complex. The constitutive activity of GMH39 irreversibly decreases during the course of repeated subcultures (Table 5), suggesting, for example, the

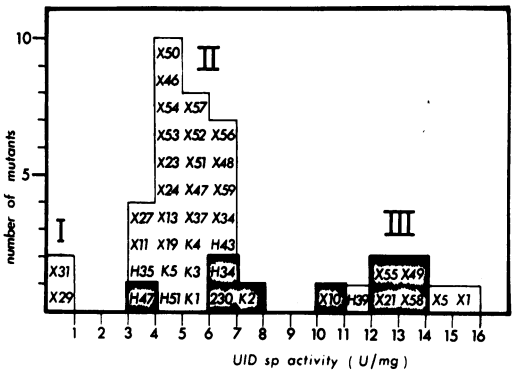

FIG. 2. *Distribution of the UID-constitutive activities of 41 MeGalU-positive mutants issued from strains: HfrH (H mutants), KL16 (K mutants), and P4X (X mutants). Pleiotropic mutants are grouped in the cross-hatched areas: GMX55 mutant from a uxuB strain was further characterized as belonging to this class (13). Units of specific activity are defined in the text.*

segregation of a duplicated structural gene. On the other hand, a very small number of transductants from strain GMX5 exhibit a weak but stable UID activity (GMS906, Table 5). It has been determined that this mutant, obtained in the presence of NTG, possessed a double, cotransducible mutation.

Inducibility of UID synthesis in specific constitutive mutants. Prior studies (9) have shown that induction of UID requires two effectors: MeGlcU (or GlcU) and fructuronate (or mannonic amide). The natural inducer is MeGlcU, which yields fructuronate by its own degradation via two enzymatic steps (Fig. 1). Induction can therefore be prevented by metabolic blocking, which was done in strains having an *uxaC* mutation, which affects uronic isomerase (Fig. 1) (9).

When the metabolic path is not blocked, MeGlcU normally induces specific constitutive mutants belonging to classes I and II (Fig. 2) up

TABLE 3. *Mapping of the constitutive mutations for UID synthesis, by P1 transduction using GMS529 (aroD,man) as the recipient strain*

Donor mutant strain	Man⁺ transductant (no.)	% Unselected phenotype		Aro⁺ transductant (no.)	% Unselected phenotype	
		pNPGlcU⁺	Aro⁺[a]		pNPGlcU⁺[a]	Man⁺[a]
GMK4	35	74	0			
GMH39	69	76	0	128	0.8	0.8
GMX29	52	77	2	47	0	0
GMX13	79	77	7	32	0	0
GMX5	72	77	0	125	4.2	3
GMX23	32	78	3	30	(10)	(7)
GMX19	58	79	3			
GMX37	74	82	1.5	25	(8)	(4)
GMX31	57	84	0	64	1.5	1.5
GMH43	64	85	0	32	(6)	(6)
GMH34	60	85	0	96	2	2
GMX34	98	85	3			
GMX42	32	85	0			
GMX24	60	85	0			
GMX27	30	87	(7)	30	(3)	0
GMX1	33	94	0	101	2	2
GMH42	31	94	0			

[a] Numbers in parentheses are statistically not significant because of the small number of clones selected.

TABLE 4. *Location of the pNPGlcU-positive phenotype in relation to uidA*

Donor P1 (pNPGlcU⁺) (*uid*⁺)	Recipient strain (pNPGlcU⁻) (*uidA1*)	Selected phenotype	No.	% Unselected phenotype[a]				
				Man⁺	Aro⁺	Uid⁺	pNPGlcU⁺	pNPGlcU⁻
GMK1	GMS407	Man⁺	110			93	89	4
		Uid⁺	109	87			100	0
	GM290	Aro⁺	165			4.2	4.2	0
		Uid⁺	108		3		96	4[b]
GMK4	GMS407	Uid⁺	29	69			93	7[c]
GMK5	GMS407	Man⁺	38			95	95	0

[a] The pNPGlcU was screened on Uid⁺ clones only.
[b] These clones, Uid⁺, pNPGlcU⁻, were Aro⁻.
[c] These clones, Uid⁺, pNPGlcU⁻, were Man⁻.

TABLE 5. *UID synthesis in some specific constitutive mutants and their derivatives*

Mutant	Derivative strain	Relevant genotype	UID sp act (U/mg)[a]	Origin of the derivative strains
GMK1			5.70	
	S468	*arg,aroD*	3.07	*man*$^+$ from GMS343 by P1 transduction from K1
	S474	*arg,trp*	0.27	*his*$^+$,*str* from a cross between PA609 and K1
	S507	*arg*	2.26	*trp*$^+$ from S474 by P1 transduction from HfrH
GMX13		*metB*	4.85	
	S661	*arg,thyA*	3.00	*man*$^+$,*aro*$^+$ from S529 by P1 transduction from X13
	S707	*arg,aroD,recA*	4.50	*man*$^+$ from S529 by P1 transduction from X13 and then *thy*$^+$,*recA* from a cross with Hfr KL16-99
GMX1		*metB*	15.89	
	S876	*metB*	14.79	*man*$^+$ from S838 by P1 transduction from X1
	S641	*arg,aroD*	14.70	*man*$^+$ from S343 by P1 transduction from X1
GMX5		*metB*	13.99	
	S901	*metB*	10.55	*man*$^+$ from S838 by P1 transduction from X5
	S906	*metB*	0.27	*man*$^+$ from S838 by P1 transduction from X5
	S887	*arg,aroD,thyA*	5.05	*man*$^+$ from S529 by P1 transduction from X5
GMH39			11.98	
			7.20	After repeated subcultures
			5.20	After repeated subcultures
			3.27	After repeated subcultures
	S883	*arg,aroD,thyA*	9.76	*man*$^+$ from S529 by P1 transduction from H39

[a] Units of specific activity are defined in the text.

to a maximal level of 6 to 7 U/mg. Glucuronate, which has a weaker inducer activity than MeGlcU in wild-type strains (9), normally induces mutants of class I, no longer induces the less-derepressed mutants of class II, and represses the more-derepressed mutants of classes II and III (Table 6).

A strong induction can be obtained by the sole addition of an inducer of the second type: fructuronate or mannonic amide (Table 6).

When the metabolic pathway is blocked by an *uxaC* mutation, neither MeGlcU nor GlcU is capable of inducing; therefore, specific constitutive mutations are only sensitive to inducers of the second type.

Expression of constitutive mutations in F-merodiploids. By crossing F-prime strain 500 (wild type) with F-minus strains carrying a constitutive mutation, merodiploid F-ductants Aro$^+$ (Pro$^+$ or Met$^+$) were selected. Although *man-4* was the most distal marker of the episome, selection of Man$^+$ clones was not used to avoid additional repression by mannose in the growth medium.

At the end of each experiment, the ability of these merodiploids to donate *uid*$^+$ or *man*$^+$ was assayed to check that the episome used was not shortened, deleted, or integrated. The results (Table 7) show that two kinds of constitutive mutants can be distinguished. (i) The expression of the constitutive mutations from strain GMX1 and from the weak mutation of the GMX5 double mutant (GMS906) was not significantly affected by the corresponding wild-type allele of the episome (residual activity, ca. 40 to 60%). Such mutations are reminiscent of the operator-constitutive (O^c) type as first defined in the lactose operon (5). (ii) In all the other mutants, UID synthesis was strongly repressed by the introduction of the wild-type allele carried by the episome (residual activity, generally 1 to 3%). This repression was fully relieved by the addition of the inducer (Table 8). The recessive character of such constitutive mutations in the presence of the wild-type allele characterizes mutations in a regulatory gene (I$^-$ type), as described for the lactose system (5).

Expression of some *uid*$^-$ mutations in F-

TABLE 6. *Inducibility of UID synthesis by MeGlcU, glucuronate, fructuronate and mannonic amide in uidR and uidR-uxaC strains*

Mutant strain	*uid* allele	*uxaC* genotype	UID sp act (U/mg)[a] in cells grown on glycerol plus:				
			None	MeGlcU[b]	GlcU[b]	FruU[b]	ManN[b]
GMX29	*R3*	+	0.31	4.95	2.56		
			0.22				6.26
GMS828[c]	*R3*	−	0.54		1.00	1.39	
			0.29	0.31			4.48
			0.18		0.45		3.91
GMX31	*R6*	+	0.91	6.00	2.89		
			0.59			1.63	
GMS829[c]	*R6*	−	0.80	0.75			
			0.71	1.03	1.09	1.60	
			0.40	0.40	0.70		5.03
GMX27	*R11*	+	2.69			2.36	6.12
			3.16	6.19	3.04		
GMX11	*R19*	+	3.84	6.76	1.66		
			5.33			4.13	7.01
GMX13	*R4*	+	4.29	7.41	2.66		
			3.80			3.92	5.98
			5.58			4.42	6.78
GMK1	*R1*	+	5.70	5.30	1.34		
			4.25	5.26	2.72	4.60	8.33
GMX5	*R10*	+	11.76	9.52	5.00		
			11.59			13.58	14.36
GMS979	*R101*	+	0.01	0.03			0.10
GMS744[d]	*R101, R21*	+	2.34	2.25	1.84		
			2.18			2.25	3.52
			3.14				6.57

[a] Units of specific activity are defined in the text.

[b] Abbreviations and concentrations used: MeGlcU, methyl-β-D-glucuronide (10 mM); GlcU, glucuronate (10 mM); FruU, fructuronate (1 mM); ManN, mannonic amide (3 mM); TPU, phenyl-thio-β-D-glucuronide (5 mM).

[c] GMS828 and GMS829 were derived stepwise as follow. First, a Met$^+$ (*arg*) transductant of AU128 (*uxaC*) was selected—strain GMS788. Next, this strain was crossed with Hfr strains GMX29 and GMX31, and then a (Uxa$^-$, pNPGlcU$^+$) recombinant was selected in each cross.

[d] For the origin of GMS744, see footnote *b* of Table 9.

TABLE 7. *Expression of UID synthesis in merodiploid strains carrying specific constitutive mutations*[a]

Primary mutant	*uid* mutation	Mutagenic treatment[b]	UID sp act (U/mg)[c]			
			Haploid *recA* strains		Diploid	
			Original F^-	Segregated F^-	1st clone	2nd clone
GMX1	*O2*		3.87		1.57	3.34
GMH39	*R9*	UV	8.94	7.11	0.29	0.19
GMX11	*R19*	NTG	7.56	2.42	0.16	0.14
GMH42	*R18*	UV	5.89	2.05	0.09	0.03
GMH43	*R8*	EMS	5.24	5.54	0.19	0.10
GMX5	*R10*	NTG	5.26	4.80	0.17	0.16
GMX13	*R4*	Sp	4.15	2.19	0.07	0.13
GMK1	*R1*	Sp	3.67	2.50	0.03	0.01
GMK4	*R23*	Sp	3.59		0.02	0.02
GMX23	*R5*	UV	3.04	2.24	0.03	0.04
GMX24	*R24*	NO_2	3.00		0.03	0.02
GMK5	*R25*	Sp	2.93		0.02	0.03
GMX19	*R26*	EMS	2.57		0.03	0.02
GMX34	*R27*	Sp	2.57		0.02	0.03
GMX27	*R11*	UV	2.25		0.02	0.03
GMH51	*R20*	Sp	2.24		0.02	0.02
GMX37	*R28*	Sp	2.17		0.02	0.03
GMX50	*R29*	Sp	2.00	0.43	0.02	0.01
GMX47	*R30*	Sp	1.70	1.06	0.02	0.02
GMX15	*R31*	Sp	1.50	0.88	0.13	0.04
GMX35	*R32*	Sp	1.43		0.03	
GMX31	*R6*	Sp	0.31	0.33	0.02	0.02
GMX29	*R3*	Sp	0.32	0.26	0.01	0.005
GMX5	*O3*	NTG	0.10	0.06	0.06	0.06

[a] Aro^+ merodiploids were selected as described in the text.
[b] Sp, Spontaneous; NO_2, sodium nitrite.
[c] Units of specific activity are defined in the text.

merodiploids. Among the uid^- mutations mapped in the *uidA* region, two (carried by strains GMN81 and GMN121) were able to revert to constitutivity for UID synthesis. These primary mutations were strongly cotransducible with *man* (92 and 85%, respectively). The reverse mutation of GMN81 was also located in this locus (87% cotransduction with *man*).

In merodiploids, negative mutations in GMN81 and GMN121 were dominant over the wild-type allele and prevented induction of UID synthesis in the presence of MeGlcU (Table 9). Moreover, the constitutive phenotype of the reverse mutant of GMN81 (strain GRS182) was repressed by the uid^+ allele, and inducibility was not fully recovered (Table 9). Such behavior appears to result from superrepressed mutations (I^s type) in a regulatory gene, as is known in the lactose (2, 5, 23) or in the galactose system (17).

TABLE 8. *Inducibility of merodiploid strains carrying specific constitutive mutations*

Strains	Mutant allele	UID sp act (U/mg)[a]		
		Haploid, uninduced	Diploid	
			Uninduced	Induced
GMK1	*uidR1*	3.67	0.01	1.89[b] 2.89[c]
GMX31	*uidR6*	0.31	0.02	7.68[c] 5.82[c]
GMX47	*uidR30*	0.64	0.01	0.84[b]

[a] Units of specific activity are defined in the text.
[b] Overnight cultures grown on glycerol were diluted in the presence of glycerol plus MeGlcU (10 mM), and cells were harvested.
[c] Cells were adapted to grow on MeGlcU (10 mM) before harvesting.

Expression of UID synthesis in strains GMX29 and GMX31. The two weak constitutive mutations were, surprisingly, found to be recessive to the wild-type allele, like the other presumed *uidR* mutations. We compared the expression of UID activity in these strains with that in fully derepressed *uidR* strains. We observed that UID extracted from all of these strains showed the same thermosensitivity secondly, that at low temperature (30 C) UID synthesis was still constitutive (Table 10), and, finally, that inducibility of UID remained unmodified in these weak *uidR* mutants (Table 6).

DISCUSSION

All of the MeGalU-positive mutants exhibit a derepressed UID synthesis. In most of them the derepression only affects this enzyme and depends only on one mutation located close to *uidA*, the UID structural gene.

Study of the expression of UID synthesis in merodiploid strains enables us to distinguish two types of mutations. The first type (strain GMX1) is dominant over the inducible wild-type allele. The *cis* dominance was evident in another mutant (13), and these results enable us to define the operator site *uidO* of gene *uidA*. The second type of mutations is present in all of the other specific constitutive mutants: the mutations are recessive to the wild-type allele (Table 7). Repression of the constitutive synthesis is specific for the mutated gene since it is relieved by the natural inducer (Table 8). Such mutations would affect a regulatory gene, *uidR*, which is specifically associated with the expression of the structural gene *uidA*.

At this locus we mapped two *uid*-negative

TABLE 9. *Expression of MeGlcU-induced UID synthesis in merodiploid strains carrying uidA-negative and uidR-superrepressed mutations*

Uid⁻ mutant	Strain	Genotype	UID sp act (U/mg)[a]	
			Uninduced	Induced
GMN81	GMS769[b]	*uidR101*		0.02
	F500/GMS769	F *uidR⁺/uidR101*		0.31
	F500/GMS769			0.22
	F500/GMS769			0.28
	F500/GMS769			0.32
GM121	GMS770[b]	*uidR102*		0.27
	F500/GMS770	F *uidR⁺/uidR102*		0.57
	F500/GMS770			0.50
	F500/GMS770			0.57
	F500/GMS770			0.40
GM291	GMS576[b]	*uidA1*		0.005
	F500/GMS576	F *uidA⁺/uidA1*		7.87
GM241	GMS560[b]	*uidA2*		0.005
	F500/GMS560	F *uidA⁺/uidA2*		1.32
	F500/GMS560			2.33
	F500/GMS560			1.28
GRS182	GMS744[b]	*uidR101, uidR21*	2.30	2.25
	F500/GMS744	F *uidR⁺/uidR101,uidR21*	0.05	1.08
			0.06	1.97
			0.11	
			0.10	

[a] Units of specific activity are defined in the text.

[b] *man⁺,uid⁻* derivative from GMS529 by P1 transduction from the indicated donors followed by the introduction of *thy⁺,rec⁻* by conjugation with KL16-99.

TABLE 10. *UID synthesis in weakly derepressed uidR mutants at various temperatures*

Strain	Genotype	UID sp act (U/mg)[a] in cultures grown on glycerol at:		
		30 C	37 C	40 C
GMX29	*uidR3*	0.26	0.48	1.19
GMX31	*uidR6*	0.57	0.91	1.93

[a] Units of specific activity are defined in the text.

mutations showing the superrepressed characteristics of a regulatory gene: (i) they are *trans* dominant over the *uid⁺* allele in merodiploid cells, and (ii) they revert to UID-specific constitutivity (*uidR*). These results confirm the existence of a regulatory gene operating via the action of a repressor molecule.

One of the important results of the present work is the behavior of two weakly derepressed mutants: GMX29 and GMX31. Surprisingly, their constitutive mutation appeared to be recessive as is that of the other *uidR* mutants.

Partially derepressed mutants that are nevertheless affected in a regulatory gene have been occasionally identified in the lactose system (6). More recently, Shineberg (18) obtained a group of mutants constitutive for β-galactosidase synthesis all of which, except one, were characterized by a mutation in the regulatory gene *lacI*. Of these, 40% were weakly derepressed (18).

As Shineberg did earlier (18), we obtained but a few operator-constitutive O^c mutants (3/37 tested) and no I^--dominant (I^{-d}) mutants. In contrast, the distribution of UID specific activity in *uidR* mutants is more dispersed than that in *lacI* mutants (18). Moreover, the mutants that are the less derepressed (class I) exhibit a derepression rate that is much higher than that of the corresponding class of *lacI* mutants: 80- to 200-fold as opposed to 3- to 4-fold the basal level of the wild-type strain used (18). This difference is probably due to the method of selection: MeGalU is a very poor substrate that does not allow growth of very weakly derepressed mutants.

Most specific mutants, in addition to pleiotropic mutants from HfrH and Hfr KL16 strains, are grouped in class II (Fig. 2).

A few specific mutants are grouped in class III along with pleiotropic mutants from P4X

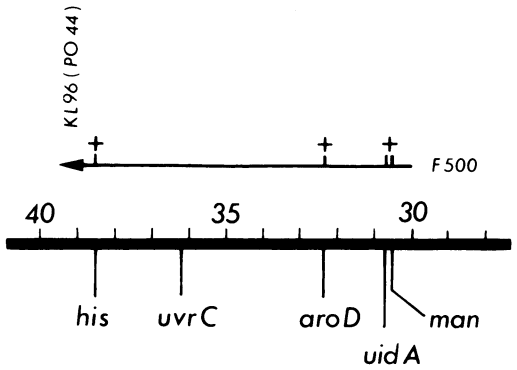

FIG. 3. *Chromosomal map of E. coli K-12 from the min 30 to min 40, showing the genes covered by the F-prime strain F500 (so named by B. J. Bachmann, Coli Genetic Stock Center). This strain was obtained from the Hfr strain KL96 (point of origin no. 44) (8).*

strain. In these, the strong derepression can be explained, in one case, by a double mutation in the control sites of gene *uidA* (GMX5) and, in another case, perhaps, by a duplication of genes (GMH39).

The most striking finding of the present study concerns the mode of induction of partially derepressed *uidR* mutants. In contrast to what happens with wild-type strains (9), induction of UID no longer requires the simultaneous presence of two types of effectors in these mutants. Mannonic amide, or (with less efficiency) fructuronate, inducers of the second type, can alone induce *uidR* mutants (Table 6).

These results suggest two conclusions. (i) The inducers of the second type must operate at the gene and not at the metabolic level (9) since mannonic amide is a component that is not metabolized by *E. coli* cells. (ii) The strains carrying a *uidR* mutation have become insensitive to the inducer effect of MeGlcU.

Consequently, the expression of *uidA* appears to be dependent on two distinct gene controls. The first control (gene $uidR^+$) would be sensitive to inducers of the first type (β-glucuronide or glucuronate); the second would be sensitive to the inducer molecules of the *uxu* operon (fructuronate or mannonic amide). The fact that we obtained MeGalU-positive mutants constitutive for the expression of both *uidA* and *uxu* operons confirms this last hypothesis (13).

ACKNOWLEDGMENTS

This work was greatly aided by grants from the Délégation Générale à la Recherche Scientifique et Technique (Biologie Moléculaire and Interactions Moléculaires en Biologie) and the Centre National de la Recherche Scientifique (Equipe de Recherche Associée no. 177; directed by F. Stoeber).

We especially thank Barbara Bachmann from the Coli Genetic Stock Center and K. B. Low for kindly providing some useful strains. We acknowledge the excellent technical collaboration of Gisèle Couchoux and J. Fontanet; F. Harper collaborated in earlier experiments.

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